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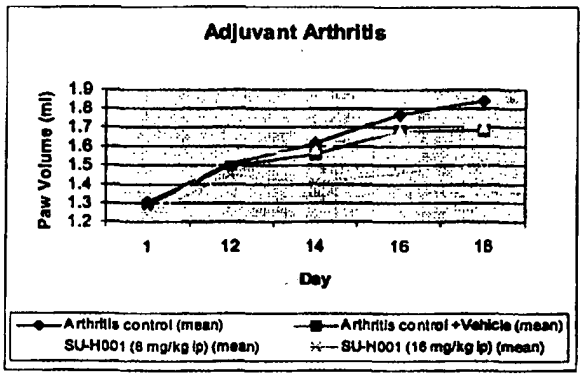
(54) Title: HETEROCYCLIC FAMILIES OF COMPOUNDS FOR THE MODULATION OF TYROSINE PROTEIN KINASE

(57) Abstract

The invention relates to certain indolinone-based and pyrazolylamide-based compounds, their method of synthesis, and combinatorial libraries consisting of the compounds. The invention also relates to methods of modulating the function of protein kinases using these compounds and methods of treating diseases by modulating the function of protein kinases and related signal transduction pathways.



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DESCRIPTION

HETEROCYCLIC FAMILIES OF COMPOUNDS FOR THE MODULATION OF TYROSINE PROTEIN KINASE

RELATED APPLICATIONS

This application is related to and claims priority to the U.S. Provisional Applications Serial No. 60/079,713, filed March 26, 1998, by Tang, et al., and entitled "METHODS OF MODULATING TYROSINE PROTEIN KINASE FUNCTION WITH TRICYCLIC-BASED INDOLINONE COMPOUNDS" (Lyon & Lyon Docket No. 231/250), Serial No. 60/081,792, filed April 15, 1998, by Tang, et al., and entitled "PYRAZOLYLAMIDE-BASED COMPOUNDS AS MEDICAMENTS" (Lyon & Lyon Docket No. 231/249), Serial No. 60/080,422, filed April 2, 1998, by Tang, et al., and entitled "METHODS OF MODULATING TYROSINE PROTEIN KINASE FUNCTION WITH INDOLINONE COMPOUNDS" (Lyon & Lyon Docket No. 231/248), Serial No. 60/082,056, filed 04/16/98 April 16, 1998, by Tang, et al., and entitled "2-INDOLINONE DERIVATIVES AS MODULATORS OF PROTEIN KINASE ACTIVITY" (Lyon & Lyon Docket No. 230/288), Serial No. 60/098,783, filed September 1, 1998, by Tang, et al., and entitled "NOVEL PHENYL 2-INDOLINONE DERVATIVES AD MODULATORS OF PROTEIN KINASE ACTIVITY" (Lyon & Lyon Docket No. 236/127), Serial No. 60/089,521, filed June 16, 1998, by Tang, et al., and entitled "METHODS FOR TREATING DISEASES AND DISORDERS RELATED TO UNREGULATED ANGIOGENESIS AND/OR VASCULOGENESIS" (Lyon & Lyon Docket No. 227/126), and Serial No. 60/089,397, filed 06/15/98 June 15, 1998, by Hannah, et al., and entitled "USE OF INDOLINONE COMPOUNDS TO ENHANCE SEXUAL ACTIVITY" (Lyon & Lyon Docket No. 234/252).

BACKGROUND OF THE INVENTION

The following description of the background of the invention is provided to aid in understanding the invention, but is not admitted to describe or constitute prior art to the invention.

Cellular signal transduction is a fundamental mechanism whereby extracellular stimuli are relayed to the interior of cells and subsequently regulate diverse cellular processes. One of the key biochemical mechanisms of signal transduction involves the

reversible phosphorylation of proteins. Phosphorylation of polypeptides regulates the activity of mature proteins by altering their structure and function. Phosphate most often resides on the hydroxyl moiety (-OH) of serine, threonine, or tyrosine amino acids in proteins.

Enzymes that mediate phosphorylation of cellular effectors generally fall into two classes. The first class consists of protein kinases which transfer a phosphate moiety from adenosine triphosphate to protein substrates. The second class consists of protein phosphatases which hydrolyze phosphate moieties from phosphoryl protein substrates. The converse functions of protein kinases and protein phosphatases balance and regulate the flow of signals in signal transduction processes.

Protein kinases and protein phosphatases are generally divided into two groups - receptor and non-receptor type proteins. Most receptor-type protein tyrosine phosphatases contain two conserved catalytic domains, each of which encompasses a segment of 240 amino acid residues. Saito *et al.*, 1991, *Cell Growth and Diff. 2*:59-65. Receptor protein tyrosine phosphatases can be subclassified further based upon the amino acid sequence diversity of their extracellular domains. Saito *et al.*, supra; Krueger *et al.*, 1992, *Proc. Natl. Acad. Sci. USA 89*:7417-7421.

Protein kinases and protein phosphatases are also typically divided into three classes based upon the amino acids they act upon. Some catalyze the addition or hydrolysis of phosphate on serine or threonine only, some catalyze the addition or hydrolysis of phosphate on tyrosine only, and some catalyze the addition or hydrolysis of phosphate on serine, threonine, and tyrosine.

Tyrosine kinases can regulate the catalytic activity of other protein kinases involved in cell proliferation. Protein kinases with inappropriate activity are also involved in some types of cancer. Abnormally elevated levels of cell proliferation are associated with receptor and non-receptor protein kinases with unregulated activity.

In addition to their role in cellular proliferation, protein kinases are thought to be involved in cellular differentiation processes. Cell differentiation occurs in some cells upon nerve growth factor (NGF) or epidermal growth factor (EGF) stimulation. Cellular differentiation is characterized by rapid membrane ruffling, cell flattening, and increases in cell adhesion. Chao, 1992, *Cell 68*:995-997.

In an effort to discover novel treatments for cancer and other diseases, biomedical researchers and chemists have designed, synthesized, and tested molecules that inhibit the function of protein kinases. Some small organic molecules form a class of compounds that modulate the function of protein kinases. Examples of molecules that have been reported to inhibit the function of protein kinases are bis-monocyclic, bicyclic or heterocyclic aryl compounds (PCT WO 92/20642), vinylene-azaindole derivatives (PCT WO 94/14808), 1-cyclopropyl-4-pyridyl-quinolones (U.S. Patent No. 5,330,992), styryl compounds (by Levitzki, et al., U.S. Patent No. 5,217,999, and entitled "Styryl Compounds which Inhibit EGF Receptor Protein Tyrosine Kinase), styryl-substituted pyridyl compounds (U.S. Patent No. 5,302,606), certain quinazoline derivatives (EP Application No. 0 566 266 A1), seleoindoles and selenides (PCT WO 94/03427), tricyclic polyhydroxylic compounds (PCT WO 92/21660), and benzylphosphonic acid compounds (PCT WO 91/15495).

The compounds that can traverse cell membranes and are resistant to acid hydrolysis are potentially advantageous therapeutics as they can become highly bioavailable after being administered orally to patients. However, many of these protein kinase inhibitors only weakly inhibit the function of protein kinases. In addition, many inhibit a variety of protein kinases and will therefore cause multiple side-effects as therapeutics for diseases.

Despite the significant progress that has been made in developing compounds for the treatment of cancer, there remains a need in the art to identify the particular structures and substitution patterns that form the compounds capable of modulating the function of particular protein kinases.

SUMMARY OF THE INVENTION

I. Overview

The present invention is directed in part towards tricyclic-based indolinone compounds, pyrazolylamide-based compounds, imidazoyl 2-indolinone derivatives, phenyl 2-indolinone derivatives, and methods of modulating the function of protein kinases (PKs) with these compounds. The methods incorporate cells that express a protein kinase. In addition, the invention describes methods of preventing and treating protein

kinase-related abnormal conditions in organisms with a compound identified by the methods described herein. Furthermore, the invention pertains to pharmaceutical compositions comprising compounds identified by methods of the invention.

The present invention features indolinone compounds that potently inhibit protein kinases and related products and methods. Inhibitors of protein kinases can be obtained by adding chemical substituents to an indolinone compound. The compounds of the invention represent a new generation of therapeutics for diseases associated with one or more functional or non-functional protein kinases. Neuro-degenerative diseases and certain types of cancer fall into this class of diseases. The compounds can be modified such that they are specific to their target or targets and will subsequently cause few side effects and thus represent a new generation of potential cancer therapeutics. These properties are significant improvements over the currently utilized cancer therapeutics that cause multiple side effects and deleteriously weaken patients.

It is believed the compounds of the invention will minimize and obliterate solid tumors by inhibiting the activity of the protein kinases, or will at least modulate or inhibit tumor growth and/or metastases. Protein kinases regulate proliferation of blood vessels during angiogenesis, among other functions. Increased rates of angiogenesis accompany cancer tumor growth in cells as cancer tumors must be nourished by oxygenated blood during growth. Therefore, inhibition of the protein kinase and the corresponding decreases in angiogenesis will starve tumors of nutrients and most likely obliterate them.

While a precise understanding of the mechanism by which compounds inhibit PKs (e.g., the fibroblast growth factor receptor 1 (FGFR1)) is not required in order to practice the present invention, the compounds are believed to interact with the amino acids of the PKs' catalytic region. PKs typically possess a bi-lobate structure, and ATP appears to bind in the cleft between the two lobes in a region where the amino acids are conserved among PKs Inhibitors of PKs are believed to bind to the PKs through non-covalent interactions such as hydrogen bonding, Van der Waals interactions, and ionic bonding, in the same general region that ATP binds to the PKs. More specifically, it is thought that the oxyindole component of the compounds of the present invention binds in the same general space occupied by the adenine ring of ATP. Specificity of an indolinone PK inhibitor for a particular PK may be conferred by interactions between the constituents around the oxyindole core with amino acid domains specific to individual PKs. Thus,

different indolinone substitutents may contribute to preferential binding to particular PKs. The ability to select those compounds active at different ATP binding sites makes them useful in targeting any protein with such a site, not only protein tyrosine kinases, but also serine/threonine kinases and protein phosphatases. Thus, such compounds have utility for *in vitro* assays on such proteins and for *in vivo* therapeutic effect through such proteins.

Unregulated blood vessel growth, due to angiogenesis and vascularization, which is at least in part responsible for diseases and disorders such as rheumatoid arthritis, endometriosis, ocular diseases, cancer and metastases, psoriasis, arterial thickening and restenosis, and excessive scarring during wound healing, is dependent on phosphorylation of substrate molecules by activated tyrosine kinases. The methods of the invention can minimize angiogenesis and vascularization of tissues by specifically inhibiting the activity of protein kinases, which regulate proliferation of blood vessels during angiogenesis. This inhibition can result in a blockade to tyrosine signal transduction and an interruption of abnormal cell proliferation and thus disease progression. Alternatively, as in the case of tissue ischemia, it may be useful to increase the activity of protein kinases in order to increase the proliferation of blood vessels through angiogenesis.

Finally, the compounds of the invention may potently inhibit the action of phosphatases and may represent a new generation of therapeutics for diseases associated with defects in said phosphatases. Thus, the compounds of the present invention may be used to modulate the functions of protein phosphatases and may be used for preventing and treating protein phosphatase related abnormal conditions in organisms. Terms defined herein with respect to kinases have a similar meaning to one skilled in the art with respect to phosphatases.

The present invention also provides for methods of preventing and treating sexual dysfunction in mammals, especially humans. The invention provides methods for administering a compound to a mammal in need thereof and monitoring the progress of the mammal's sexual activity, including, in males, erectile function, the ability to perform intercourse, maintaining an erection subsequent to penetration, and orgasm, and in females, increased vaginal lubrication and orgasm.

II. Definitions

The term "compound" refers to the compound or a pharmaceutically acceptable salt, ester, amide, prodrug, isomer, or metabolite, thereof.

Different compounds that have the same molecular formula are called "isomers." Isomers have the same numbers of the same kinds of atoms, but the atoms are attached to one another in different ways. Thus, all stereoisomers of the indolinone compounds are within the scope of the invention.

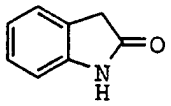
The term "pharmaceutically acceptable salt" refers to a formulation of a compound that does not abrogate the biological activity and properties of the compound. Pharmaceutical salts can be obtained by reacting a compound of the invention with inorganic or organic acids such as hydrochloric acid, hydrobromic acid, sulfuric acid, nitric acid, phosphoric acid, methanesulfonic acid, ethanesulfonic acid, *p*-toluenesulfonic acid, salicylic acid and the like.

A "prodrug" refers to an agent that is converted into the parent drug *in vivo*. Prodrugs are often useful because, in some situations, they may be easier to administer than the parent drug. They may, for instance, be bioavailable by oral administration whereas the parent drug is not. The prodrug may also have improved solubility in pharmaceutical compositions over the parent drug. An example, without limitation, of a prodrug would be a compound of the present invention which is administered as an ester (the "prodrug") to facilitate transmittal across a cell membrane where water solubility is detrimental to mobility but which then is metabolically hydrolyzed to the carboxylic acid, the active entity, once inside the cell where water solubility is beneficial.

A further example of a prodrug might be a short polypeptide, for example, without limitation, a 2 - 10 amino acid polypeptide, bonded through a terminal amino group to a carboxy group of a compound of this invention wherein the polypeptide is hydrolyzed or metabolized *in vivo* to release the active molecule.

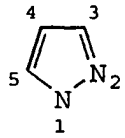
The term "indolinone" is used as that term is commonly understood in the art and includes a large subclass of substituted or unsubstituted compounds that are capable of being synthesized from an aldehyde moiety and a oxindole moiety.

The term "oxindole" refers to an oxindole compound optionally substituted with chemical substituents. Oxindole compounds are of the general structure:



The term "pyrazolylamide" is used as that term is commonly understood in the art and includes a large subclass of substituted or unsubstituted compounds that are formed by linking an aryl moiety with a pyrazol moiety through an amide bond, where such linking takes place at position 3 of the pyrazol ring.

The term "pyrazol" refers to a pyrazol compound substituted with chemical substituents. Pyrazol compounds are of the general structure:



The term "substituted", in reference to the invention, refers to a compound that is derivatized with any number of chemical substituents.

The term "alkyl" refers to saturated or unsaturated alkyl optionally substituted with substituents selected from the group consisting of halogen, trihalomethyl, carboxylate, amino, nitro, ester, and a five-membered or six-membered aromatic, heteroaromatic, aliphatic, or heteroaliphatic ring moiety, where the ring moiety is optionally substituted with one, two, or three substituents independently selected from the group consisting of alkyl, halogen, trihalomethyl, carboxylate, amino, nitro, and ester moieties.

The term "saturated alkyl" refers to an alkyl moiety that does not contain any alkene or alkyne moieties. The alkyl moiety may be branched or non-branched.

The term "unsaturated alkyl" refers to an alkyl moiety that contains at least one alkene or alkyne moiety. The alkyl moiety may be branched or non-branched.

A "cycloalkyl" group refers to an all-carbon monocyclic or fused ring (i.e., rings which share an adjacent pair of carbon atoms) group wherein one or more of the rings does not have a completely conjugated pi-electron system. Examples, without limitation, of cycloalkyl groups are cyclopropane, cyclobutane, cyclopentane, cyclopentene, cyclohexane, cyclohexadiene, cycloheptane and, cycloheptatriene. A cycloalkyl group may be substituted or unsubstituted. When substituted, the substituent group(s) is preferably one or more individually selected from alkyl, aryl, heteroaryl, heteroalycyclic,

hydroxy, alkoxy, aryloxy, thiohydroxy, thioalkoxy, thioaryloxy, cyano, halo, carbonyl, thiocarbonyl, carboxy, O-carbamyl, N-carbamyl, C-amido, N-amido, nitro, amino and NR10R11 with R_{10} and R_{11} as previously defined herein.

An "alkenyl" group refers to an alkyl group, as defined herein, consisting of at least two carbon atoms and at least one carbon-carbon double bond.

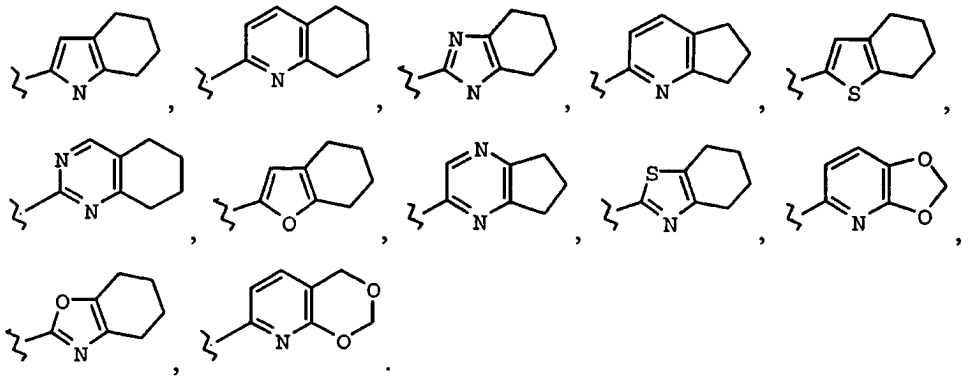
An "alkynyl" group refers to an alkyl group, as defined herein, consisting of at least two carbon atoms and at least one carbon-carbon triple bond.

The term "aromatic" refers to an aromatic group which has at least one ring having a conjugated pi electron system and includes both carbocyclic aryl (e.g. phenyl) and heterocyclic aryl groups (e.g. pyridine). The term "carbocyclic" refers to a compound which contains one or more covalently closed ring structures, and that the atoms forming the backbone of the ring are all carbon atoms. The term thus distinguishes carbocyclic from heterocyclic rings in which the ring backbone contains at least one atom which is different from carbon. The term "heteroaromatic" refers to an aromatic group which contains at least one heterocyclic ring. The aromatic or heteroaromatic rings defined herein may optionally be substituted with one, two, or three substituents independently selected from the group consisting of alkyl, alkoxy, halogen, trihalomethyl, carboxylate, amino, nitro, and ester moieties.

The term "aliphatic ring" refers to a compound which contains one or more covalently closed ring structures, and that at least one of the atoms forming the backbone is a saturated carbon atom (e.g. cyclohexane). The term "heteroaliphatic ring" refers to a ring system in which at least one of the atoms forming the backbone is a heteroatom (e.g. tetrahydropyran). The aliphatic or heteroaliphatic rings defined herein may optionally be substituted with one, two, or three substituents independently selected from the group consisting of alkyl, alkoxy, halogen, trihalomethyl, carboxylate, amino, nitro, ester, and an aromatic or heteroaromatic ring optionally substituted with one, two, or three substituents independently selected from the group consisting of alkyl, alkoxy, halogen, trihalomethyl, carboxylate, amino, nitro, and ester moieties.

A "fused heteroaryl:cycloalkyl/heteroalicyclic" group refers to a heteroaryl group in which two adjacent carbon atoms of the heteroaryl ring are connected to one another, in addition to the direct covalent bond of each of them to the heteroaryl ring, by a five-atom or a six-atom chain which may consist of all carbon atoms or carbon atoms combined with

heteroatoms such as nitrogen, oxygen and or sulfur; this second connection results in the formation of a second ring. Examples, without limitation, of fused heteroaryl:cycloalkyl/heteroalicyclic groups are:



The fused heteroaryl:cycloalkyl/heteroalicyclic groups may be unsubstituted or substituted. When substituted, the substitutent(s) on a heteroaryl moiety of a fused heteroaryl:cycloalkyl/heteroalicyclic group are selected from those set forth above in the definition of the heteroaryl group. Likewise, when substituted, the substituents on a cycloalkyl or a heteroalicyclic moiety of a fused heteroaryl:cycloalkyl/hetero- alicyclic group are selected from those set forth in the definitions of cycloalkyl and heteroalicyclic.

The term "amine" refers to a chemical moiety of formula $-(X_1)_{n1}-NX_2X_3$, where X_1 is selected from the group consisting of saturated or unsaturated alkyl, and five-membered or six-membered aromatic, heteroaromatic, or aliphatic ring moieties and where n1 is 0, 1, or 2, and where X_2 and X_3 are independently selected from the group consisting of hydrogen, saturated or unsaturated alkyl, five-membered or six-membered aromatic, heteroaromatic, or aliphatic ring moieties, and where X_2 and X_3 together form a five-membered or six-membered heteroaromatic or heteroaliphatic ring, as those rings are defined herein.

The term "halogen" refers to an atom selected from the group consisting of fluorine, chlorine, bromine, and iodine. The term "trihalomethyl" refers to the $-CX_3$ group, where X is a halogen. A "trihalomethanesulfonyl" group refers to a $X_3CS(=O)_2-$ groups where X is a halogen.

The term "ketone" refers to a chemical moiety with formula $-(X_4)_{n4}-CO-X_5$, where X_4 and X_5 are independently selected from the group consisting of alkyl optionally substituted with halogen, trihalomethyl, carboxylate, amino, nitro, ester, and five-

membered or six-membered aromatic, heteroaromatic, aliphatic, or heteroaliphatic ring moieties, where the ring moieties are optionally substituted with one, two, or three substituents independently selected from the group consisting of alkyl, halogen, trihalomethyl, carboxylate, amino, nitro, and ester moieties and where n4 is 0, 1, or, 2.

The term "carboxylic acid" refers to a chemical moiety with formula $-(X_6)_{n6}-COOH$. The term "ester" refers to a chemical moiety with formula $-(X_7)_{n7}-COO-X_8$. X_6, X_7, and X_8 and are independently selected from the group consisting of alkyl and five-membered or six-membered aromatic, heteroaromatic, aliphatic, or heteroaliphatic ring moieties and where n6 and n7 are independently 0, 1, or, 2.

An "O-carboxy" group refers to a $X_4C(=O)O-$ group, with X_4 as defined herein.

A "C-carboxy" group refers to a $-C(=O)OX_4$ group, with X_4 as defined herein.

A "thiocarbonyl" group refers to a $-C(=S)-X_4$ group, with X_4 as defined herein.

The term "alcohol" refers to a chemical substituent of formula $-(X_9)_{n9}-OH$, and the term "alkoxyalkyl" refers to a chemical substituent of formula $-(X_{10})_{n10}-O-X_{11}$, where X_9, X_{10}, and X_{11} are independently selected from the group consisting of saturated or unsaturated alkyl, and five-membered or six-membered aromatic, heteroaromatic, aliphatic, or heteroaliphatic ring moieties, where the ring is optionally substituted with one or more substituents independently selected from the group consisting of alkyl, halogen, trihalomethyl, carboxylate, nitro, and ester and where n9 and n10 are independently 0, 1, or, 2. When n is 0, then the alkoxyalkyl moiety is called an "alkoxy moiety".

The term "alkoxyalkoxy" refers to a chemical substituent of formula $-O-(X_{20})_{n20}-O-X_{23}$, where X_{20} and X_{23} are each independently selected from the group consisting of alkyl, five-membered or six-membered aromatic, heteroaromatic, aliphatic, or heteroaliphatic ring moieties optionally substituted with one or more substituents independently selected from the group consisting of alkyl, halogen, trihalomethyl, carboxylate, nitro, or ester, and where n20 is 0 or 1.

The term "amide" refers to a chemical substituent of formula $-(X_{12})_{n12}-NHCOX_{13}$, i.e., "N-amido", or of formula $-(X_{14})_{n14}-CONX_{15}X_{16}$, i.e., "C-amido", where X_{12} and X_{14} are each independently selected from the group consisting of alkyl, and five-membered or six-membered aromatic, heteroaromatic, aliphatic, or heteroaliphatic ring moieties, where the ring is optionally substituted with one or more substituents independently selected from the group consisting of alkyl, halogen, trihalomethyl, carboxylate, nitro, and ester

and where n12 and n14 are independently 0, 1, or, 2, and where X_{13}, X_{15}, and X_{16} are each independently selected from the group consisting of hydrogen, alkyl, hydroxyl, and five-membered or six-membered aromatic, heteroaromatic, aliphatic, or heteroaliphatic ring moieties, where the ring is optionally substituted with one or more substituents independently selected from the group consisting of alkyl, halogen, trihalomethyl, carboxylate, nitro, and ester.

The term "cyano" refers to a chemical moiety with formula -C≡N.

The term "cyanato" refers to a chemical moiety with formula -CNO.

The term "isocyanato" refers to a chemical moiety with formula -NCO.

The term "thiocyanato" refers to a chemical moiety with formula -CNS.

The term "isothiocyanato" refers to a chemical moiety with formula -NCS.

The term "sulfonamide" refers to a chemical moiety with formula $-(X_{17})_{n17}-SO_2NX_{18}X_{19}$, where X_{18}, and X_{19} are independently selected from the group consisting of alkyl, five-membered or six-membered aromatic, heteroaromatic, aliphatic, or heteroaliphatic ring moieties optionally substituted with one or more substituents independently selected from the group consisting of alkyl, halogen, trihalomethyl, carboxylate, nitro, or ester, and where X_{18} and X_{19} taken together form a five-membered or six-membered aliphatic or heteroaliphatic ring optionally substituted with one or more substituents independently selected from the group consisting of alkyl, halogen, trihalomethyl, carboxylate, amino, nitro, and ester, and X_{17} is selected from the group consisting of alkyl, five-membered or six-membered aromatic, heteroaromatic, aliphatic, or heteroaliphatic ring moieties optionally substituted with one or more substituents independently selected from the group consisting of alkyl, halogen, trihalomethyl, carboxylate, nitro, or ester, and where X_{18} and X_{19} taken together form a five-membered or six-membered aliphatic or heteroaliphatic ring optionally substituted with one or more substituents independently selected from the group consisting of alkyl, halogen, trihalomethyl, carboxylate, amino, nitro, and ester, where n17 is 0, 1, or, 2.

The term "aldehyde" refers to a chemical moiety with formula $-(X_{20})_{n20}-CO-H$ where X_{20} is selected from the group consisting of saturated or unsaturated alkyl, and five-membered or six-membered aromatic, heteroaromatic, aliphatic, or heteroaliphatic ring moieties, where the ring is optionally substituted with one or more substituents

independently selected from the group consisting of alkyl, halogen, trihalomethyl, carboxylate, amino, nitro, and ester and where n20 is 0, 1, or, 2.

The term "sulfone" refers to a chemical moiety with formula $-(X_{21})_{n21}-SO_2-X_{22}$, X_{21} and X_{22} are independently selected from the group consisting of saturated or unsaturated alkyl, and five-membered or six-membered aromatic, heteroaromatic, aliphatic, or heteroaliphatic ring moieties, where the ring is optionally substituted with one or more substituents independently selected from the group consisting of alkyl, halogen, trihalomethyl, carboxylate, amino, nitro, and ester and where n21 is 0, 1, or, 2.

A "sulfinyl" group refers to a $-S(=O)-X_{21}$ group where X_{21}, in addition to being as previously defined, may also be a hydroxy group.

The term "thiol" refers to a chemical moiety with formula$-(X_{23})_{n23}-SH$, and the term "thioether" refers to a chemical moiety of the formula $-(X_{24})_{n24}-S-X_{25}$, where X_{23}, X_{24}, and X_{25} are independently selected from the group consisting of saturated or unsaturated alkyl, and five-membered or six-membered aromatic, heteroaromatic, aliphatic, or heteroaliphatic ring moieties, where the ring is optionally substituted with one or more substituents independently selected from the group consisting of alkyl, halogen, trihalomethyl, carboxylate, amino, nitro, and ester and where n23 and n24 are independently 0, 1, or, 2.

The term "acyl" refers to chemical moieties of the general formula -C(O)R. When R is hydrogen the molecule containing the acyl group is an aldehyde. When R is an alkyl, an aliphatic ring, or an aromatic ring, then the molecule containing the acyl group is a ketone.

The term "O-carbamyl" refers to a chemical moiety with formula $-OC(=O)NR_{10}R_{11}$ group with R_{10} and R_{11} as defined herein.

The term "N-carbamyl" group refers to a chemical moiety with formula $R_{10}OC(=O)NR_{11}-$, with R_{10} and R_{11} as defined herein.

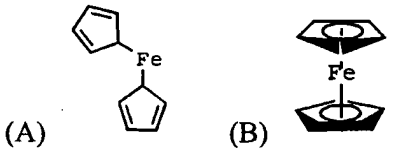
The term "O-thiocarbamyl" group refers to a chemical moiety with formula $-OC(=S)NR_{10}R_{11}$ with R_{10} and R_{11} as defined herein.

The term "N-thiocarbamyl" group refers to a chemical moiety with formula $R_{11}OC(=S)NR_{10}-$, with R_{10} and R_{11} as defined herein.

The term "heavy metal" refers to elements in the transition metal series of the periodic table of the elements, and include elements number 21-30, 39-48, and 57-82. The

heavy metal may be substituted with a five-membered or six-membered aromatic or heteroaromatic ring moieties, where the ring is optionally substituted with one or more substituents independently selected from the group consisting of alkyl, halogen, trihalomethyl, carboxylate, amino, nitro, and ester.

The term "ferrocene" refers to an organometallic sandwich complex containing an atom of iron sandwiched by two cyclopentadienyl groups. The cyclopentadienyl rings can be optionally substituted with substituents described herein. Ferrocene is normally depicted using one of the two structures shown below, structure A and structure B.



(A) (B)

By "combined" or "combined to form a 5- or 6-member ring" when referring to two adjacent "R" groups attached to a ring system, is meant that, the two R groups are linked together by one or two additional atoms; i.e., the structure becomes {-R-X-X-R-} such that a 5- or 6-member ring is formed. X can be carbon, nitrogen, oxygen or sulfur.

A "combinatorial library" refers to all the compounds formed by the reaction of each compound in one dimension of a multi-dimensional array with a compound in each of the other dimensions of the multi-dimensional array. As used herein, the multi-dimensional array is two dimensional, one dimension being all the oxindoles of this invention and the other dimension being all the aldehydes of this invention. Each oxindole may be reacted with each of the aldehydes to form a 2-indolinone. All 2-indolinone compounds formed in this manner are within the scope of this invention. Also within the scope of this invention are smaller combinatorial libraries formed by the reaction of some of the oxindoles of this invention with all the aldehydes of this invention or all of the oxindoles with some of the aldehydes or some of the oxindoles with some of the aldehydes.

By "about" in the context of set temperatures, i.e. "about 50 °C" or "about 90 °C", it is meant that the temperature range is preferably ±10 °C, more preferably ±5 °C, and most preferably ±2 °C. Thus, for example, by "about 50 °C", it is meant that the

temperature range is preferably 50±10 °C, more preferably 50±5 °C, and most preferably 50±2 °C.

The term "pharmaceutical composition" refers to a mixture of an indolinone compound of the invention with other chemical components, such as diluents, excipients, or carriers. The pharmaceutical composition facilitates administration of the compound to an organism. Multiple techniques of administering a compound exist in the art including, but not limited to, oral, injection, aerosol, parenteral, and topical administration. Pharmaceutical compositions can also be obtained by reacting compounds with inorganic acids such as hydrochloric acid, hydrobromic acid, sulfuric acid, nitric acid, phosphoric acid, methanesulfonic acid, ethanesulfonic acid, *p*-toluenesulfonic acid, salicylic acid and the like.

The term "pharmaceutically acceptable" or "pharmaceutical" as used herein refers to solutions or components of the formulation that do not prevent the therapeutic compound from exerting a therapeutic effect and do not cause unacceptable adverse side effects. Examples of pharmaceutically acceptable reagents are provided in *The United States Pharmacopeia The National Formulary*, United States Pharmacopeial Convention, Inc., Rockville, MD 1990 (hereby incorporated by reference herein, including any drawings, figures, or tables). Unacceptable side effects vary for different diseases. Generally, the more severe the disease the more toxic the effects that will be tolerated. Unacceptable side effects for different diseases are known in the art.

The term "carrier" defines a chemical compound that facilitates the incorporation of a compound into cells or tissues. For example dimethyl sulfoxide (DMSO) is a commonly utilized carrier as it facilitates the uptake of many organic compounds into the cells or tissues of an organism.

The term "diluent" defines chemical compounds diluted in water that will dissolve the compound of interest as well as stabilize the biologically active form of the compound. Salts dissolved in buffered solutions are utilized as diluents in the art. One commonly used buffered solution is phosphate buffered saline because it mimics the salt conditions of human blood. Since buffer salts can control the pH of a solution at low concentrations, a buffered diluent rarely modifies the biological activity of a compound.

The term "function" refers to the cellular role of a protein kinase. The protein kinase family includes members that regulate many steps in signaling cascades, including

cascades controlling cell growth, migration, differentiation, gene expression, muscle contraction, glucose metabolism, cellular protein synthesis, and regulation of the cell cycle.

The term "catalytic activity", in the context of the invention, defines the rate at which a protein kinase phosphorylates a substrate. Catalytic activity can be measured, for example, by determining the amount of a substrate converted to a product as a function of time. Phosphorylation of a substrate occurs at the active-site of a protein kinase. The active-site is normally a cavity in which the substrate binds to the protein kinase and is phosphorylated.

The term "substrate" as used herein refers to a molecule phosphorylated by a protein kinase. The substrate is preferably a peptide and more preferably a protein.

The term "activates" refers to increasing the cellular function of a protein kinase. The protein kinase function is preferably the interaction with a natural binding partner and most preferably catalytic activity.

The term "inhibit" refers to decreasing the cellular function of a protein kinase. The protein kinase function is preferably the interaction with a natural binding partner and most preferably catalytic activity.

The term "modulates" refers to altering the function of a protein kinase by increasing or decreasing the probability that a complex forms between a protein kinase and a natural binding partner. Such modulation may take place either *in vivo* or *in vitro*. A modulator preferably increases the probability that such a complex forms between the protein kinase and the natural binding partner, more preferably increases or decreases the probability that a complex forms between the protein kinase and the natural binding partner depending on the concentration of the compound exposed to the protein kinase, and most preferably decreases the probability that a complex forms between the protein kinase and the natural binding partner. A modulator preferably activates the catalytic activity of a protein kinase, more preferably activates or inhibits the catalytic activity of a protein kinase depending on the concentration of the compound exposed to the protein kinase, or most preferably inhibits the catalytic activity of a protein kinase.

The term "complex" refers to an assembly of at least two molecules bound to one another. Signal transduction complexes often contain at least two protein molecules bound to one another.

The term "natural binding partner" refers to polypeptides that bind to a protein kinase in cells. Natural binding partners can play a role in propagating a signal in a protein kinase signal transduction process. A change in the interaction between a protein kinase and a natural binding partner can manifest itself as an increased or decreased probability that the interaction forms, or an increased or decreased concentration of the protein kinase/natural binding partner complex.

A protein kinase natural binding partner can bind to a protein kinase's intracellular region with high affinity. High affinity represents an equilibrium binding constant on the order of 10^{-6} M or less. In addition, a natural binding partner can also transiently interact with a protein kinase intracellular region and chemically modify it. Protein kinase natural binding partners are chosen from a group that includes, but is not limited to, *SRC* homology 2 (SH2) or 3 (SH3) domains, other phosphoryl tyrosine binding (PTB) domains, guanine nucleotide exchange factors, protein phosphatases, and other protein kinases. Methods of determining changes in interactions between protein kinases and their natural binding partners are readily available in the art.

The term "contacting" as used herein refers to mixing a solution comprising a compound of the invention or other compounds such as VEGF, FGF, or PDGF, with a liquid medium bathing the cells of the methods. The solution comprising the compound may also comprise another component, such as dimethylsulfoxide (DMSO), which facilitates the uptake of the indolinone compound or compounds into the cells of the methods. The solution comprising the indolinone compound may be added to the medium bathing the cells by utilizing a delivery apparatus, such as a pipet-based device or syringe-based device.

The term "monitoring" refers to observing the effect of adding the compound to the cells of the method. The effect can be manifested in a change in cell phenotype, cell proliferation, protein kinase catalytic activity, or in the interaction between a protein kinase and a natural binding partner. The term "monitoring" is further used herein in reference to an effect on rats in the adjuvant arthritis model to include general disease symptoms including ear nodulation, tail nodulation, nose swelling, paw swelling, and balanitis. An arthritis index can be calculated from these measurements as defined in the Examples section.

The term "effect" describes a change or an absence of a change in cell phenotype or cell proliferation. "Effect" can also describe a change or an absence of a change in the catalytic activity of the protein kinase. "Effect" can also describe a change or an absence of a change in an interaction between the protein kinase and a natural binding partner.

The term "cell phenotype" refers to the outward appearance of a cell or tissue or the function of the cell or tissue. Examples of cell phenotype are cell size (reduction or enlargement), cell proliferation (increased or decreased numbers of cells), cell differentiation (a change or absence of a change in cell shape), cell survival, apoptosis (cell death), or the utilization of a metabolic nutrient (*e.g.*, glucose uptake). Changes or the absence of changes in cell phenotype are readily measured by techniques known in the art.

The term "antibody" refers to an antibody (*e.g.*, a monoclonal or polyclonal, antibody), or antibody fragment, having specific binding affinity to protein kinase or its fragment.

By "specific binding affinity" is meant that the antibody binds to target (protein kinase) polypeptides with greater affinity than it binds to other polypeptides under specified conditions. Antibodies having specific binding affinity to a protein kinase may be used in methods for detecting the presence and/or amount of a protein kinase in a sample by contacting the sample with the antibody under conditions such that an immunocomplex forms and detecting the presence and/or amount of the antibody conjugated to the protein kinase. Diagnostic kits for performing such methods may be constructed to include a first container containing the antibody and a second container having a conjugate of a binding partner of the antibody and a label, such as, for example, a radioisotope. The diagnostic kit may also include notification of an FDA approved use and instructions therefor.

The term "polyclonal" refers to antibodies that are heterogenous populations of antibody molecules derived from the sera of animals immunized with an antigen or an antigenic functional derivative thereof. For the production of polyclonal antibodies, various host animals may be immunized by injection with the antigen. Various adjuvants may be used to increase the immunological response, depending on the host species.

"Monoclonal antibodies" are substantially homogenous populations of antibodies to a particular antigen. They may be obtained by any technique which provides for the

production of antibody molecules by continuous cell lines in culture. Monoclonal antibodies may be obtained by methods known to those skilled in the art. See, for example, Kohler, et al., *Nature* 256:495-497 (1975), and U.S. Patent No. 4,376,110.

The term "antibody fragment" refers to a portion of an antibody, often the hypervariable region and portions of the surrounding heavy and light chains, that displays specific binding affinity for a particular molecule. A hypervariable region is a portion of an antibody that physically binds to the polypeptide target.

The term "aberration", in conjunction with a signal transduction process, refers to a protein kinase that is over- or under-expressed in an organism, mutated such that its catalytic activity is lower or higher than wild-type protein kinase activity, mutated such that it can no longer interact with a natural binding partner, is no longer modified by another protein kinase or protein phosphatase, or no longer interacts with a natural binding partner.

The term "promoting or disrupting the abnormal interaction" refers to a method that can be accomplished by administering a compound of the invention to cells or tissues in an organism. A compound can promote an interaction between a protein kinase and natural binding partners by forming favorable interactions with multiple atoms at the complex interface. Alternatively, a compound can inhibit an interaction between a protein kinase and natural binding partners by compromising favorable interactions formed between atoms at the complex interface.

The term "preventing" as used herein refers to administering a composition to a patient before an abnormal condition manifests itself in that patient, and at least partially stopping or reducing the abnormal condition that would otherwise result.

The term "treating" as used herein refers to the method of the invention having a therapeutic effect and at least partially alleviating or abrogating an abnormal condition in the organism.

The term "therapeutic effect" as used herein refers to the inhibition of cell growth causing or contributing to an abnormal condition. The term "therapeutic effect" also refers to the inhibition of factors causing or contributing to the abnormal condition. A therapeutic effect also refers to relieving to some extent one or more of the symptoms of the abnormal condition.

The term "rheumatoid arthritis" as used herein refers to a chronic systemic disease primarily of the joints, usually polyarticular, marked by inflammatory changes in the synovial membranes and articular structures and by muscle atrophy and rarefaction of the bones. Forms of rheumatoid arthritis include, but are not limited to, juvenile, chronic villous, cricoarytenoid, deformans, degenerative, mutilans, and proliferative.

The term "endometriosis" as used herein refers to a condition in which tissue containing typical endometrial granular and stromal elements occurs aberrantly in various locations in the pelvic cavity or some other area of the body (most commonly the peritoneal cavity).

The term "ocular disease" as used herein refers to diseases of, pertaining to, or affecting the eye, specifically those where new capillaries in the retina invade the vitreous, bleed, and can cause blindness. Examples include, but are not limited to, senile macular degeneration and diabetic retinopathy.

The term "cancer and metastases" as used herein refers to a new growth of tissue in which the multiplication of cells is uncontrolled and progressive and a growth of abnormal cells distant from the site primarily involved by the morbid process.

The term "psoriasis" as used herein refers to a common chronic, squamous dermatosis with polygenic inheritance and a fluctuating course. Methods of diagnosis are well-known to those in the art. It is a chronic skin disorder characterized by hyperproliferation of the epidermis, inflammation and angiogenesis.

The term "arterial thickening" as used herein refers to thickening of the arterial wall as part of the atherosclerotic process or the result of treatments for coronary occlusions, for example. Atherosclerosis refers to an extremely common form of arteriosclerosis in which deposits of yellowish plaques are formed in arteries.

The term "restenosis" as used herein refers to recurrent stenosis, especially of a valve of the heart, after surgical correction of the primary condition. Stenosis refers to narrowing or stricture of a duct or canal.

The term "tissue ischemia" as used herein refers to a deficiency of blood in tissue, usually due to a functional constriction or actual obstruction of a blood vessel.

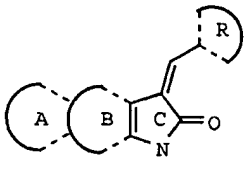
The term "excessive scarring during wound healing" as used herein refers to the result of uncontrolled angiogenesis leading to neovascularization during wound healing.

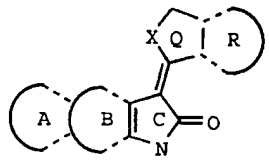
An example is keloid formation. A keloid is a sharply elevated, irregularly-shaped, progressively enlarging scar.

III. Tricyclic-Oxindole-Based Indolinone Compounds

A. Compounds

In a first aspect, the invention features a tricyclic-based indolinone compound having a structure set forth in formula I or II:

(I) 

(II) 

where

(a) ring A and ring B share one common bond;

(b) ring B and ring C share one common bond;

(c) ring A, ring B, and ring R are independently selected from the group consisting of an aromatic ring, a heteroaromatic ring, an aliphatic ring, a heteroaliphatic ring, and a fused aromatic or aliphatic ring system, where the heteroaromatic ring and heteroaliphatic ring each independently contain 0, 1, 2, or 3 heteroatoms independently selected from the group consisting of nitrogen, oxygen, and sulfur; and

(d) ring A, ring B, ring Q, and ring R are each independently and optionally substituted with one, two, or three substituents independently selected from the group consisting of (i) alkyl; (ii) an aromatic or heteroaromatic ring; (iii) an aliphatic or heteroaliphatic ring; (iv) an amine; (v) a nitro of formula $-NO_2$; (vi) a halogen or trihalomethyl; (vii) a ketone; (viii) a carboxylic acid or ester; (ix) an alcohol or an alkoxyalkyl moiety; (x) an amide; (xi) a sulfonamide; (xii) an aldehyde; (xiii) a sulfone; (xiv) a thiol or a thioether; and (xv) a heavy metal; and

(e) X is selected from the group consisting of CH and oxygen.

In preferred embodiments, ring A and ring B of the tricyclic-based indolinone compound of formula I are each independently selected from the group consisting of a 5-

membered ring, a 6-membered ring, a 7-membered ring, and an 8-membered ring. More preferably, ring A and ring B are each a 6-membered ring.

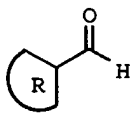
In another preferred embodiment, ring R in formula I is selected from the group consisting of a 5-membered ring, a 6-membered ring, a 7-membered ring, an 8-membered ring, and a bicyclic or tricyclic fused ring system. More preferably, R is a 5-membered ring, even more preferably, R is a 6-membered ring. In a preferred embodiment, Ring R is a bicyclic fused ring system comprising preferably 8, more preferably 9, and even more preferably 10, and most preferably 13 atoms in the ring backbone. In other preferred embodiments, R is an optionally substituted ferrocene.

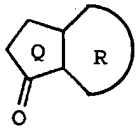
Ring R is most preferrably derived from an aldehyde, ketone, or lactone selected from the group consisting of the compounds set forth in Table 8.

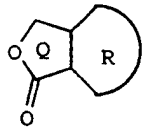
In another aspect, the invention features a library, *e.g.*, a combinatorial library of at least 10 tricyclic-based indolinone compounds that can be formed by reacting an oxindole with an aldehyde, ketone, or lactone, where the oxindole has a structure set forth in formula III

(III) 

and where the aldehyde has a structure set forth in formula IV, where the ketone has a structure set forth in formula V, and where the lactone has a structure set forth in formula VI

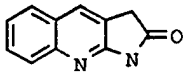
(IV) 

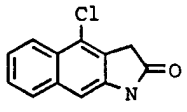
(V) 

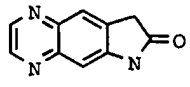
(VI) 

where rings A, B, Q, and R are as defined herein or any of the subgroups thereof set forth herein.

The oxindole of the combinatorial library preferably has a structure set forth in formula VII, VIII, or IX:

(VII) 

(VIII) 

(IX) 

where each ring in said formulae is optionally substituted with one or more substituents independently selected from the group consisting of (i) saturated or unsaturated alkyl; (ii) an aromatic or heteroaromatic ring; (iii) an aliphatic or heteroaliphatic ring; (iv) an amine of formula $-(X_1)_{n1}-NX_2X_3$, where X_1, X_2, and X_3 are independently selected from the group consisting of hydrogen, saturated or unsaturated alkyl, and five-membered or six-membered aromatic, heteroaromatic, or aliphatic ring moieties and where n1 is 0, 1, or 2; (v) a nitro of formula $-NO_2$; (vi) a halogen or trihalomethyl; and (vii) an alcohol of formula $-(X_9)_{n9}-OH$ or an alkoxyalkyl moiety of formula $-(X_{10})_{n10}-O-X_{11}$, where X_9, X_{10}, and X_{11} are independently saturated or unsaturated alkyl and where n9 and n10 are independently 0 or 1.

The aldehyde, ketone, or lactone of the combinatorial library is selected from the group consisting of the compounds set forth in Table 8.

B. Synthesis

In a further aspect, the invention features a method for synthesizing a tricyclic-based indolinone compound comprising the steps of:

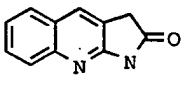
(a) reacting a first reactant with a second reactant in a solvent and in the presence of a base at elevated temperatures, where the first reactant is an oxindole, and where the second reactant is an aldehyde;

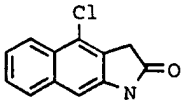
(b) purifying said tricyclic-based indolinone compound.

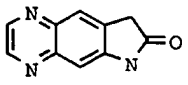
The first reactant preferably is an oxindole having the structure set forth in formula III:

(III) 

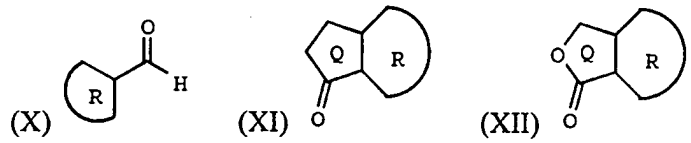
where rings A, B, and C are as defined herein or any of the subgroups thereof set forth herein. Most preferably, the oxindole has the structure set forth in formula VII, VIII, or IX:

(VII) 

(VIII) 

(IX) 

The second reactant is preferably an aldehyde, ketone, or lactone, where the aldehyde has a structure set forth in formula X, the ketone has a structure set forth in formula XI, and the lactone has a structure set forth in formula XII



where rings Q and R in the above structures are as defined herein or any of the subgroups thereof set forth herein. Most preferably, the second reactant is an aldehyde, ketone, or lactone selected from the group consisting of the compounds set forth in Table 8.

The base is preferably a nitrogen base, and most preferably, the base is piperidine.

"Nitrogen bases" are commonly used in the art and are selected from acyclic and cyclic amines. Examples of nitrogen bases include, but are not limited to, ammonia, methyl amine, trimethylamine, aniline, and piperidine. Those skilled in the art know which nitrogen base would match the requirements of the reaction conditions.

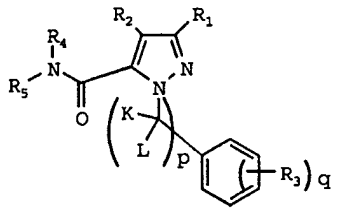
The solvent of the reaction is preferably an alcohol or an amide, and most preferably, the solvent is ethanol or dimethylformamide (DMF).

The synthetic method of the invention calls for the reaction to take place at elevated temperatures. The term "elevated temperatures" refers to temperatures that are greater than room temperature. More preferably, the elevated temperature is about 50 °C, and most preferably the elevated temperature is about 90 °C. This method may be accompanied by the step of screening a library for a compound of the desired activity and structure - thus, providing a method of synthesis of a compound by first screening for a compound having the desired properties and then chemically synthesizing that compound.

IV. Pyrazolylamide-Based Compounds

A. Compounds

In another aspect, the invention provides for a pyrazolylamide-based compound having a structure set forth in formula X:



(X)

where

(a) R_1 and R_2 are independently selected from the group consisting of (i) hydrogen; (ii) alkyl; (iii) an aromatic or heteroaromatic ring; (iv) an aliphatic or heteroaliphatic ring; (v) an amine; (vi) a nitro of formula $-NO_2$; (vii) a halogen; (viii) a ketone; (ix) a carboxylic acid or ester; (x) an alcohol or an alkoxyalkyl moiety; (xi) an amide; (xii) a sulfonamide; (xiii) an alkoxyalkoxy; and (xiv) a sulfone; and

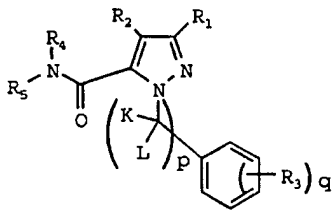
(b) R_4 and R_5 are each independently selected from the group consisting of (i) hydrogen; (ii) alkyl; (iii) an aromatic ring; (iv) a heteroaromatic ring; (v) an aliphatic or heteroaliphatic ring; (vi) an amine; (vii) a ketone; (viii) a carboxylic acid or ester; (ix) an alcohol or an alkoxyalkyl moiety; (x) an amide; (xi) a sulfonamide; (xii) a sulfone; and (xiii) an alkoxyalkoxy; and

(c) R_3 is selected from the group consisting of (i) hydrogen; (ii)alkyl; (iii)an aromatic or heteroaromatic ring; (iv) an aliphatic or heteroaliphatic ring; (v) a halogen and trihalomethyl; (vi) an amine; (vii) an amide; (viii) an alcohol or an alkoxyalkyl moiety; (ix) a carboxylic acid or ester; (x)a cyano of formula -CN; and (xi) a sulfonamide;

(d) p and q are each independently 0, 1, 2, or 3; and

(e) K and L are each independently selected from the gruop consisting of (i) hydrogen; (ii) alkyl; and (iii) K and L taken together form a three-membered, four-membered, five-membered, or six-membered aliphatic ring.

In yet another aspect, the invention provides for a pyrazolylamide-based compound having a structure set forth in formula X:



(X)

where

(a) R_1, R_4, and R_5 are independently selected from the group consisting of (i) hydrogen; (ii) alkyl; (iii) an aromatic or heteroaromatic ring; (iv) an aliphatic or heteroaliphatic ring; (v) an amine; (vi) a nitro of formula -NO_2; (vii) a halogen; (viii) a ketone; (ix) a carboxylic acid or ester; (x) an alcohol or an alkoxyalkyl moiety; (xi) an amide of formula; (xii) a sulfonamide; (xiii) an alkoxyalkoxy; and (xiv) a sulfone; and where at least one of R_4 and R_5 is an aromatic or heteroaromatic ring, as described herein; and

(b) R_2 is selected from the group consisting of (i) alkyl; (ii) an aromatic or heteroaromatic ring; (iii) an aliphatic or heteroaliphatic ring; (iv) an amine; (v) a ketone; (vi) a carboxylic acid or ester; (vii) an alcohol or an alkoxyalkyl moiety; (viii) an amide; (ix) a sulfonamide; and (x) a sulfone; and

(c) R_3 is selected from the group consisting of (i) hydrogen; (ii) alkyl; (iii) an aromatic or heteroaromatic ring; (iv) an aliphatic or heteroaliphatic ring; and (v) a halogen and trihalomethyl; (vi) an amine; (vii) an amide; (viii) an alcohol or an alkoxyalkyl moiety; (ix) a carboxylic acid or ester; (x) a cyano of formula -CN; and (xi) a sulfonamide;

(d) p and q are each independently 0, 1, 2, or 3; and

(e) K and L are each independently selected from the gruop consisting of (i) hydrogen; (ii) alkyl; and (iii) K and L taken together form a three-membered, four-membered, five-membered, or six-membered aliphatic ring.

In preferred embodiments, the invention provides for a pyrazolylamide based compound where R_1 is selected from the group consisting of (i) hydrogen; (ii) alkyl; (iii) an aromatic or heteroaromatic ring; and (iv) an aliphatic or heteroaliphatic ring.

More preferably, R_1 is selected from the group consisting of hydrogen and optionally substituted saturated or unsaturated alkyl. Most preferably, R_1 is selected from the group consisting of hydrogen, methyl, *n*-propyl, *i*-propyl, *n*-butyl, isobutyl, *sec*-butyl, and *t*-butyl. Thus, in certain preferred embodiments, R_1 is methyl, whereas in certain other preferred embodiments R_1 is *n*-propyl, and still in other preferred embodiments R_1 is *t*-butyl.

The R_2 group of the pyrazolylamide-based compounds of the invention is preferably selected from the group consisting of hydrogen, alkyl, and halogen. More preferably, the R_2 group is selected from the group consisting of hydrogen and halogen; it is most preferably hydrogen, and even most preferably bromine.

In the arylpryazoleamide-based compounds of the invention R_4 and R_5 are each independently selected preferably from the group consisting of hydrogen, alkyl, an aromatic or heteroaromatic ring, and an aliphatic or heteroaliphatic ring. Most preferably, R_4 and R_5 are each independently selected from the group consisting of hydrogen and an aromatic or heteroaromatic ring optionally substituted with one, two, or three substituents independently selected from the group consisting of alkyl and alkoxy moieties. Even most preferably, R_4 and R_5 are each independently selected from the group consisting of hydrogen, methyl, phenyl, pyridin-2-yl, pyridin-3-yl, pyridin-4-yl, 3-methyl-pyridin-2-yl, 4-methyl-pyridin-2-yl, 5-trifluoromethyl-pyridin-2-yl, 5-trifluoromethyl-pyridin-3-yl, 4,6-dimethyl-pyridin-3-yl, 2,6-dimethoxy-pyridin-3-yl, 2,3,5,6-tetrafluoro-pyridin-4-yl, quinolin-3-yl, 3-methyl-quinolin-4-yl, isoquinolin-1-yl, isoquinolin-3-yl, benzo[1,3]dioxol-5-yl, 4-methoxy-biphenyl-3-yl, 6-methoxy-biphenyl-3-yl, 6,3'-dimethoxy-biphenyl-3-yl, 9-oxo-9H-fluoren-1-yl, 9-oxo-9H-fluoren-3-yl, 7-acetylamino-9-oxo-9H-fluoren-2-yl, 2'-hydroxy-[1,1';3',1"]terphenyl-5'-yl, 9-ethyl-9H-carbazol-3-yl, and 6-oxo-6H-benzo[c]chromen-2-yl.

In certain other preferred embodiments, R_4 and R_5 are each independently selected from the group consisting of 2-trifluoromethyl-phenyl, 3-trifluoromethyl-phenyl, and 4-trifluoromethyl-phenyl.

In other preferred embodiments, the R_3 of the pyrazolylamide-based compound is selected from the group consisting of hydrogen, alkyl, halogen, and trihalomethyl.

R_3 is preferably selected from the group consisting of hydrogen, methyl, and halogen. More preferably, R_3 is hydrogen, even more preferably R_3 is methyl, and most preferably R_3 is chlorine.

In certain preferred embodiments, p and q are each independently 0, 1, 2, or 3. More preferably, p is 1, and most preferably q is 1.

In preferred embodiments, the pyrazolylamide-based compounds of the invention are selected from the group consisting of the pyrazolylamide-based compounds listed in Table 10, below, and numbered AP-001 through AP-033.

TABLE 10

Compound No.	Chemical name
AP-001	2-Benzyl-5-tert-butyl-2H-pyrazole-3-carboxylic acid (4-trifluoromethyl-phenyl)-amide
AP-002	2-Benzyl-5-tert-butyl-2H-pyrazole-3-carboxylic acid quinolin-3-ylamide
AP-003	2-Benzyl-5-tert-butyl-2H-pyrazole-3-carboxylic acid (2,6-dimethoxy-pyridin-3-yl)-amide
AP-004	2-Benzyl-5-tert-butyl-2H-pyrazole-3-carboxylic acid (2,3,5,6-tetrafluoro-pyridin-4-yl)-amide
AP-005	2-Benzyl-5-tert-butyl-2H-pyrazole-3-carboxylic acid (3-methyl-quinolin-4-yl)-amide
AP-006	2-Benzyl-5-tert-butyl-2H-pyrazole-3-carboxylic acid (4,6-dimethyl-pyridin-3-yl)-amide
AP-007	2-Benzyl-5-tert-butyl-2H-pyrazole-3-carboxylic acid benzo[1,3]dioxol-5-ylamide
AP-008	2-Benzyl-5-tert-butyl-2H-pyrazole-3-carboxylic acid (3-trifluoromethyl-phenyl)-amide
AP-009	2-Benzyl-5-tert-butyl-2H-pyrazole-3-carboxylic acid (2-trifluoromethyl-phenyl)-amide
AP-010	2-Benzyl-5-tert-butyl-2H-pyrazole-3-carboxylic acid pyridin-2-ylamide
AP-011	2-Benzyl-5-tert-butyl-2H-pyrazole-3-carboxylic acid isoquinolin-1-ylamide
AP-012	2-Benzyl-5-tert-butyl-2H-pyrazole-3-carboxylic acid pyridin-4-ylamide
AP-013	2-Benzyl-5-tert-butyl-2H-pyrazole-3-carboxylic acid pyridin-3-ylamide
AP-014	2-Benzyl-5-tert-butyl-2H-pyrazole-3-carboxylic acid (4-methyl-pyridin-2-yl)-amide
AP-015	2-Benzyl-5-tert-butyl-2H-pyrazole-3-carboxylic acid (3-methyl-pyridin-2-yl)-amide
AP-016	2-Benzyl-5-tert-butyl-2H-pyrazole-3-carboxylic acid (5-trifluoromethyl-pyridin-2-yl)-amide
AP-017	2-Benzyl-5-tert-butyl-2H-pyrazole-3-carboxylic acid isoquinolin-3-ylamide
AP-018	2-Benzyl-5-tert-butyl-2H-pyrazole-3-carboxylic acid (5-trifluoromethyl-pyridin-3-yl)-amide
AP-019	2-Benzyl-5-tert-butyl-2H-pyrazole-3-carboxylic acid (4-methoxy-biphenyl-3-yl)-amide
AP-020	2-Benzyl-5-tert-butyl-2H-pyrazole-3-carboxylic acid (9-oxo-9H-fluoren-3-yl)-amide
AP-021	2-Benzyl-5-tert-butyl-2H-pyrazole-3-carboxylic acid (7-acetylamino-9-oxo-9H-fluoren-2-yl)-amide
AP-022	2-Benzyl-5-tert-butyl-2H-pyrazole-3-carboxylic acid (6-methoxy-biphenyl-3-yl)-amide
AP-023	2-Benzyl-5-tert-butyl-2H-pyrazole-3-carboxylic acid (2'-hydroxy-[1,1';3',1"]terphenyl-5'-yl)-amide
AP-024	2-Benzyl-5-tert-butyl-2H-pyrazole-3-carboxylic acid (9-ethyl-9H-carbazol-3-yl)-amide
AP-025	2-Benzyl-5-tert-butyl-2H-pyrazole-3-carboxylic acid (9-oxo-9H-fluoren-1-yl)-amide
AP-026	2-Benzyl-5-tert-butyl-2H-pyrazole-3-carboxylic acid (6-oxo-6H-benzo[c]chromen-2-yl)-amide
AP-027	2-Benzyl-5-tert-butyl-2H-pyrazole-3-carboxylic acid biphenyl-3-ylamide
AP-028	2-Benzyl-5-tert-butyl-2H-pyrazole-3-carboxylic acid (6-methoxy-biphenyl-3-yl)-amide
AP-029	2-Benzyl-5-tert-butyl-2H-pyrazole-3-carboxylic acid (6,3'-dimethoxy-biphenyl-3-yl)-amide
AP-030	5-Methyl-2-(4-methyl-benzyl)-2H-pyrazole-3-carboxylic acid (4-trifluoromethyl-phenyl)-amide
AP-031	5-Methyl-2-(4-methyl-benzyl)-2H-pyrazole-3-carboxylic acid (3-trifluoromethyl-phenyl)-amide

Compound No.	Chemical name
AP-032	5-Methyl-2-(4-chloro-benzyl)-2H-pyrazole-3-carboxylic acid (4-trifluoromethyl-phenyl)-amide
AP-033	5-Methyl-2-(4-chloro-benzyl)-2H-pyrazole-3-carboxylic acid (3-trifluoromethyl-phenyl)-amide

The structures of the above compounds are shown in Table 11, below, where the substituents R_1, R_2, and R_3 refer to the generic structure below.

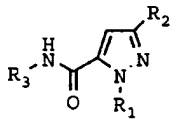


TABLE 11

Compound No.	R_1	R_2	R_3
AP-001	Benzyl	tert-butyl	4-trifluoromethyl-phenyl
AP-002	Benzyl	tert-butyl	quinolin-3-yl
AP-003	Benzyl	tert-butyl	2,6-dimethoxy-pyridin-3-yl
AP-004	Benzyl	tert-butyl	2,3,5,6-tetrafluoro-pyridin-4-yl
AP-005	Benzyl	tert-butyl	3-methyl-quinolin-4-yl
AP-006	Benzyl	tert-butyl	4,6-dimethyl-pyridin-3-yl
AP-007	Benzyl	tert-butyl	benzo[1,3]dioxol-5-yl
AP-008	Benzyl	tert-butyl	3-trifluoromethyl-phenyl
AP-009	Benzyl	tert-butyl	2-trifluoromethyl-phenyl
AP-010	Benzyl	tert-butyl	pyridin-2-yl
AP-011	Benzyl	tert-butyl	isoquinolin-1-yl
AP-012	Benzyl	tert-butyl	pyridin-4-yl
AP-013	Benzyl	tert-butyl	pyridin-3-yl
AP-014	Benzyl	tert-butyl	4-methyl-pyridin-2-yl
AP-015	Benzyl	tert-butyl	3-methyl-pyridin-2-yl
AP-016	Benzyl	tert-butyl	5-trifluoromethyl-pyridin-2-yl
AP-017	Benzyl	tert-butyl	isoquinolin-3-yl
AP-018	Benzyl	tert-butyl	5-trifluoromethyl-pyridin-3-yl
AP-019	Benzyl	tert-butyl	4-methoxy-biphenyl-3-yl
AP-020	Benzyl	tert-butyl	9-oxo-9H-fluoren-3-yl
AP-021	Benzyl	tert-butyl	7-acetylamino-9-oxo-9H-fluoren-2-yl
AP-022	Benzyl	tert-butyl	6-methoxy-biphenyl-3-yl
AP-023	Benzyl	tert-butyl	2'-hydroxy-[1,1';3',1"]terphenyl-5'-yl

Compound No.	R_1	R_2	R_3
AP-024	Benzyl	tert-butyl	9-ethyl-9H-carbazol-3-yl
AP-025	Benzyl	tert-butyl	9-oxo-9H-fluoren-1-yl
AP-026	Benzyl	tert-butyl	6-oxo-6H-benzo[c]chromen-2-yl
AP-027	Benzyl	tert-butyl	biphenyl-3-yl
AP-028	Benzyl	tert-butyl	6-methoxy-biphenyl-3-yl
AP-029	Benzyl	tert-butyl	6,3'-dimethoxy-biphenyl-3-yl
AP-030	4-methyl-benzyl	methyl	4-trifluoromethyl-phenyl
AP-031	4-methyl-benzyl	methyl	3-trifluoromethyl-phenyl
AP-032	4-methyl-benzyl	methyl	4-trifluoromethyl-phenyl
AP-033	4-methyl-benzyl	methyl	3-trifluoromethyl-phenyl

B. Synthesis

In another aspect, the invention features a method for synthesizing a pyrazolylamide-based compound comprising the steps of:

(a) reacting a first reactant with a second reactant and a base in a solvent at a set temperature; and

(b) purifying the product.

The first reactant is preferably a substituted pyrazole, and more preferably is selected from the group consisting of 1-benzyl-3-tert-butylpyrazole-5-carbonyl chloride, 1-benzyl-3-tert-butylpyrazole-5-carboxylic acid, 1-(4-methylphenyl)-3-methylpyrazole-5-carboxylic acid, 1-(4-methylbenzyl)-3-methylpyrazole-5-carboxylic acid, and 1-(4-chlorobenzyl)-3-methylpyrazole-5-carboxylic acid.

The second reactant is preferably an amine, and more preferably is selected from the group consisting of 2-trifluoromethyl-aniline, 3-trifluoromethyl-aniline, 4-trifluoromethyl-aniline, 2-aminopyridine, 3-aminopyridine, 4-aminopyridine, 2-amino-3-methyl-pyridine, 2-amino-4-methyl-pyridine, 2-amino-5-trifluoromethyl-pyridine, 3-amino-5-trifluoromethyl-pyridine, 3-amino-4,6-dimethyl-pyridine, 3-amino-2,6-dimethoxy-pyridine, 4-amino-2,3,5,6-tetrafluoro-pyridine, 3-amino-quinolin, 4-amino-3-methyl-quinolin, 1-amino-isoquinolin, 3-amino-isoquinolin, 5-amino-benzo[1,3]dioxol, 3-amino-4-methoxy-biphenyl, 3-amino-6-methoxy-biphenyl, 3-amino-6,3'-dimethoxy-biphenyl, 1-amino-9-oxo-9H-fluorene, 3-amino-9-oxo-9H-fluorene, 2-amino-7-

acetylamino-9-oxo-9H-fluorene, 5'-amino-2'-hydroxy-[1,1';3',1"]terphenyl, 3-amino-9-ethyl-9H-carbazole, and 2-amino-6-oxo-6H-benzo[c]chromene.

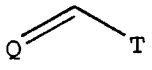
The base is preferably an amine, and more preferably it is diisopropylamine. The solvent is preferably a polar solvent, and more preferably it is selected from the group consisting of methylene chloride and dimethylformamide.

It is preferable that the reaction takes place at a set temperature. The set temperature preferably is room temperature, more preferably the temperature is higher than room temperature. When the temperature is higher than room temperature, it is preferably about 50 °C, more preferably about 55 °C, and most preferably about 90 °C.

V. Tricyclic-Aldehyde-Based Indolinone Compounds

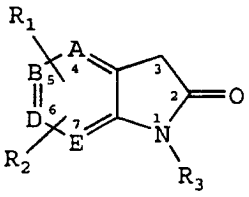
A. Compounds

In another aspect, the invention provides an indolinone compound having a structure set forth in formula XI:

(XI) 

where

(A) Q is an oxindole moiety having the structure set forth in formula XIII:

(XIII) 

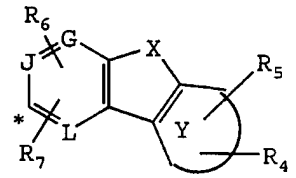
where

(a) R_1, R_2, and R_3 are independently selected from the group consisting of (i) hydrogen; (ii) alkyl; (iii) an aromatic or heteroaromatic ring; (iv) an aliphatic or heteroaliphatic; (v) an amine; (vi) a nitro of formula $-NO_2$; (vii) a halogen or trihalomethyl; (viii) a ketone; (ix) a carboxylic acid or an ester; (x) an alcohol or an alkoxyalkyl moiety; (xi) an amide; (xii) a sulfonamide; (xiii) an aldehyde; and (xiv) a sulfone; and (xv) a thiol or a thioether;

(b) A, B, D, and E are selected from the group consisting of carbon and nitrogen; and

(c) Q is bonded with the rest of the molecule through position 3 of the oxindole ring, as set forth in formula II; and

(B) T is a ring moiety having the structure set forth in formula XII:



(XII)

where

(a) R_4, R_5, R_6, and R_7 are independently selected from the group consisting of (i) hydrogen; (ii) alkyl; (iii) an aromatic or heteroaromatic ring; (iv) an aliphatic or heteroaliphatic ring; (v) an amine; (vi) a nitro of formula $-NO_2$; (vii) a halogen or trihalomethyl; (viii) a ketone; (ix) a carboxylic acid or an ester; (x) an alcohol or an alkoxyalkyl moiety; (xi) an amide; (xii) a sulfonamide; (xiii) an aldehyde; (xiv) a sulfone; and (xv) a thiol or a thioether; and

(b) X is selected from the group consisting of NX_{26}, sulfur, SO, SO_2, and oxygen, where X_{26} is selected from the group consisting of (i) hydrogen; (ii) alkyl; (iii) an aryl optionally substituted with one, two, or three substituents independently selected from the group consisting of alkyl, alkoxy, halogen, trihalomethyl, carboxylate, nitro, and ester moieties; (iv) a sulfone of formula $-SO_2-X_{27}$, where X_{27} is selected from the group consisting of saturated or unsaturated alkyl and five-membered or six-membered aryl or heteroaryl moieties; and (v) an acyl of formula $-C(O)X_{28}$, where X_{28} is selected from the group consisting of hydrogen, saturated and unsaturated alkyl, aryl, and a five-membered or six-membered ring moiety;

(c) ring Y is selected from the group consisting of five-membered, six-membered, and seven-membered aromatic, heteroaromatic, or non-aromatic rings, where the heteroaromatic ring contains a heteroatom selected from the group consisting of nitrogen, oxygen, and sulfur, and where the non-aromatic ring in combination with R_4 optionally forms a carbonyl functionality;

(d) G, J, and L are selected from the group consisting of carbon and nitrogen; and

(e) T is bonded with the rest of the molecule through position of the ring marked with an asterisk (*), as set forth in formula XII.

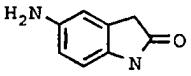
In preferred embodiments, the invention relates to an indolinone compound of formula XI, where R_1 and R_2 are selected from the group consisting of (i) hydrogen; (ii) saturated alkyl optionally substituted with halogen, trihalomethyl, carboxylate, nitro, ester, and an aliphatic or heteroaliphatic ring optionally substituted with halogen, trihalomethyl, carboxylate, nitro, and ester moieties; (iii) an aromatic ring optionally substituted with one, two, or three substituents independently selected from the group consisting of alkyl, alkoxy, halogen, and nitro moieties; (iv) an amine of formula $-(X_1)_{n1}-NX_2X_3$, where X_1 is optionally substituted saturated alkyl and where n1 is 0 or 1, and where X_2, and X_3 are independently selected from the group consisting of hydrogen and optionally substituted saturated alkyl; (v) a nitro of formula $-NO_2$; (vi) a halogen or trihalomethyl; (vii) a ketone of formula $-(X_4)_{n4}-CO-X_5$, where X_4 and X_5 are alkyl and where n4 is 0 or 1; (viii) a carboxylic acid of formula $-(X_6)_{n6}-COOH$ or ester of formula $-(X_7)_{n7}-COO-X_8$, where X_6, X_7, and X_8 are alkyl and where n6 and n7 are independently 0 or 1; (ix) an alcohol of formula $-(X_9)_{n9}-OH$ or an alkoxyalkyl moiety of formula $-(X_{10})_{n10}-O-X_{11}$, where X_9, X_{10}, and X_{11} are saturated alkyl and where n9 and n10 are independently 0 or 1; (x) an amide; and (xi) a sulfonamide.

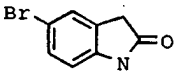
More preferably, R_1 and R_2 of the indolinone of the invention are selected from the group consisting of (i) hydrogen; (ii) methyl, ethyl, propyl, and butyl groups optionally substituted with halogen, trihalomethyl, cyano, and nitro moieties; (iii) phenyl optionally substituted with one, two, or three substituents independently selected from the group consisting of alkyl, alkoxy, halogen, and nitro moieties; (iv) an amine of formula $-(X_1)_{n1}-NX_2X_3$, where X_2 and X_3 are independently selected from the group consisting of hydrogen and optionally substituted saturated alkyl, and X_1 is optionally substituted saturated alkyl, and where n is 0 or 1; (v) a nitro of formula $-NO_2$; (vi) a halogen or trihalomethyl; (vii) a ketone of formula $-CO-X_4$, where X_4 is selected from the group consisting of methyl, ethyl, propyl, and butyl; (viii) a carboxylic acid of formula $-(X_6)_{n6}-COOH$ or ester of formula $-(X_7)_{n7}-COO-X_8$, where X_6 and X_7 are selected from the group consisting of a bond, methylene, ethylene, and propylene, and where X_8 is selected

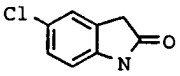
from the group consisting of methyl and ethyl, and where n6 and n7 are independently 0 and 1; (ix) an alkoxy moiety of formula -O-X_{11}, where X_{11} is selected from the group consisting of methyl and ethyl; (x) an amide of formula -NHCOX_{13}, where X_{13} is phenyl optionally substituted with one or more substituents independently selected from the group consisting of alkyl, halogen, carboxylate, or ester; and (xi) a sulfonamide of formula -$SO_2NX_{18}X_{19}$, where X_{18} and X_{19} are independently selected from the group consisting of hydrogen, methyl, ethyl, phenyl optionally substituted with one or more substituents independently selected from the group consisting of alkyl, halogen, and trihalomethyl, and where X_{18} and X_{19} taken together form a six-membered heteroaliphatic ring moiety.

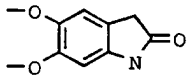
In some embodiments, E in formula XIII is nitrogen.

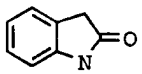
Most preferably, the indolinone compounds of the invention are those whose structure is set forth in formula XI, where Q is selected from the group consisting of type Q oxindoles. By "type Q oxindoles" is meant oxindole compounds which are selected from the list of oxindoles numbered O-1 through O-60, and are depicted as follows.

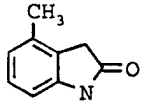
(O-1) 

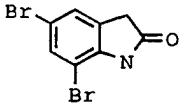
(O-2) 

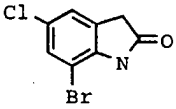
(O-3) 

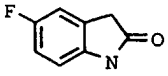
(O-4) 

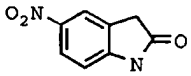
(O-5) 

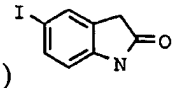
(O-6) 

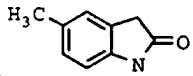
(O-7) 

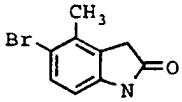
(O-8) 

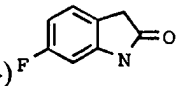
(O-9) 

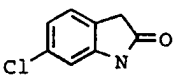
(O-10) 

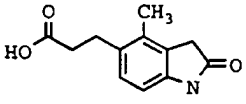
(O-11) 

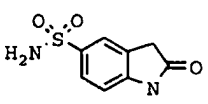
(O-12) 

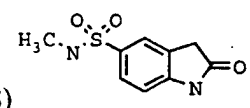
(O-13) 

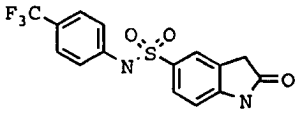
(O-14) 

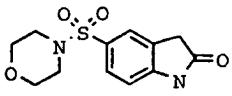
(O-15) 

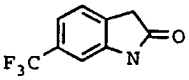
(O-16) 

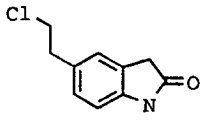
(O-17) 

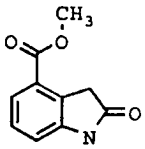
(O-18) 

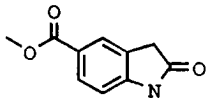
(O-19) 

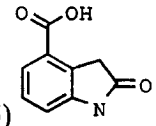
(O-20) 

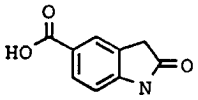
(O-21) 

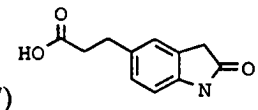
(O-22) 

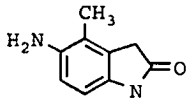
(O-23) 

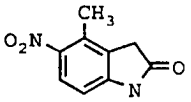
(O-24) 

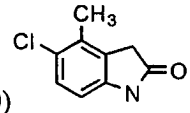
(O-25) 

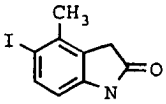
(O-26) 

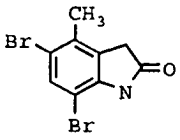
(O-27) 

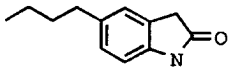
(O-28) 

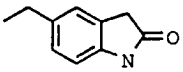
(O-29) 

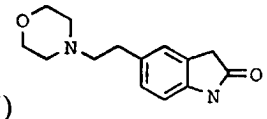
(O-30) 

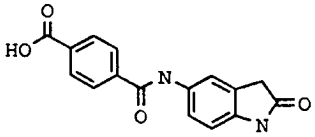
(O-31) 

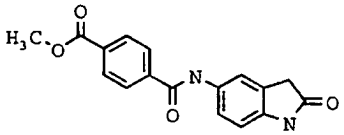
(O-32) 

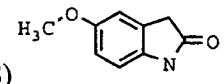
(O-33) 

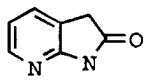
(O-34) 

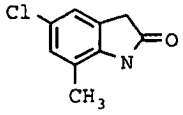
(O-35) 

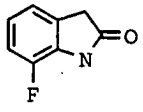
(O-36) 

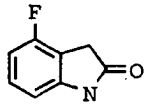
(O-37) 

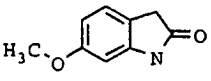
(O-38) 

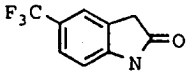
(O-39) 

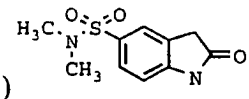
(O-40) 

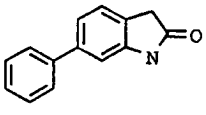
(O-41) 

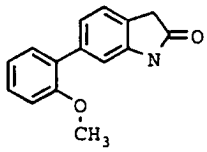
(O-42) 

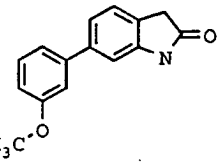
(O-43) 

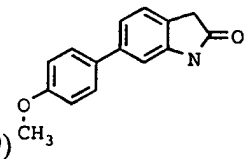
(O-44) 

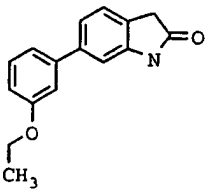
(O-45) 

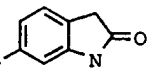
(O-46) 

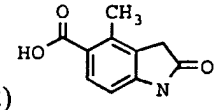
(O-47) 

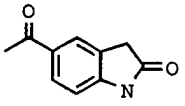
(O-48) 

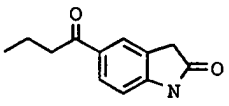
(O-49) 

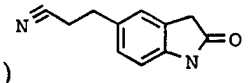
(O-50) 

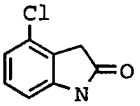
(O-51) 

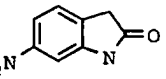
(O-52) 

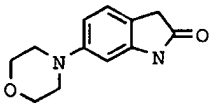
(O-53) 

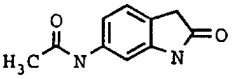
(O-54) 

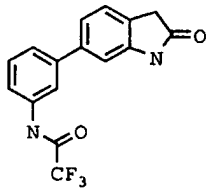
(O-55) 

(O-56) 

(O-57) 

(O-58) 

(O-59) 

(O-60) 

In certain other preferred embodiments, the indolinone of the invention has a structure as set forth in formula XI, where R_4 and R_5 are independently selected from the group consisting of

(i) hydrogen;

(ii) methyl, ethyl, propyl, and butyl groups optionally substituted with halogen, trihalomethyl, cyano, and nitro moieties;

(iii) an amine of formula $-(X_1)_{n1}-NX_2X_3$, where X_2 and X_3 are independently selected from the group consisting of hydrogen and substituted saturated alkyl, and X_1 is substituted saturated alkyl, and where n1 is 0 or 1, or where X_2 and X_3 taken together form a five-membered or a six-membered aliphatic or heteroaliphatic ring, optionally substituted at a ring carbon atom or heteroatom with a substituent selected from the group consisting of methyl, ethyl, propyl, phenyl, and alkoxyphenyl;

(iv) a nitro of formula $-NO_2$;

(v) a halogen or trihalomethyl;

(vi) a ketone of formula $-CO-X_4$, where X_4 is selected from the group consisting of methyl, ethyl, propyl, *n*-butyl, and *t*-butyl;

(vii) a carboxylic acid of formula $-(X_6)_{n6}-COOH$ or ester of formula $-(X_7)_{n7}-COO-X_8$, where X_6 and X_7 are selected from the group consisting of a bond, methylene, ethylene, and propylene, and where X_8 is selected from the group consisting of methyl and ethyl, and where n6 and n7 are independently 0 or 1;

(viii) an amide of formula $-NHCOX_{13}$, or of formula $-CONX_{15}X_{16}$, where X_{13}, X_{15}, and X_{16} are each independently selected from the group consisting of hydrogen, methyl, ethyl, propyl, and phenyl;

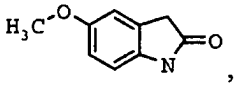
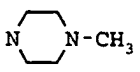
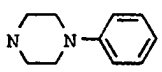
(ix) $-SO_2NX_{18}X_{19}$, where X_{18} and X_{19} are independently selected from the group consisting of hydrogen, methyl, and ethyl;

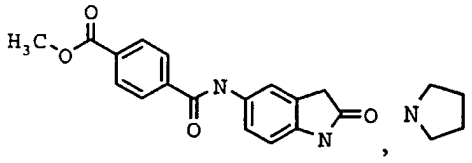
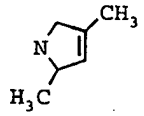
(x) an alcohol of formula $-(X_9)_{n9}-OH$ or an alkoxyalkyl moiety of formula $-(X_{10})_{n10}-O-X_{11}$, where X_9, and X_{10} are independently selected form the group consisting of methylene, ethylene, and propylene, and where X_{11} is independently selected from the group consisting of methyl, ethyl, and propyl, and where n9 and n10 are independently 0 or 1;

(xi) a sulfone of formula $-(X_{21})_{n21}-SO_2-X_{22}$, where X_{22} is selected from the group consisting of hydroxide, saturated or unsaturated alkyl, and five-membered or six-membered aryl or heteroaryl moieties, and where X_{21} is saturated alkyl, and where n21 is 0 or 1; and

(x) a thioether of formula $-(X_{24})_{n24}-S-X_{25}$, where X_{24} is independently selected from the group consisting of methylene, ethylene, and propylene, and where X_{25} is independently selected from the group consisting of methyl, ethyl, propyl, and phenyl, and where n24 is 0 or 1.

More preferably, R_4 and R_5 are each independently selected from the group consisting of (i) hydrogen; (ii) methyl and ethyl; (iii) an amine of formula $-(X_1)_{n1}-NX_2X_3$, where X_2 and X_3 are independently selected from the group consisting of hydrogen methyl and ethyl, and X_1 is methylene or ethylene, and where n1 is 0 or 1, or where X_2 and X_3 taken together form a substituted ring selected from the group consisting of

 ,  ,  ,


, and 
;

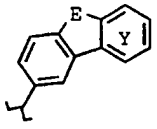
(iv) a nitro of formula $-NO_2$; (v) a halogen; (vi) a ketone of formula $-CO-X_4$, where X_4 is selected from the group consisting of methyl and *t*-butyl; (vii) a carboxylic acid of formula $-(X_6)_{n6}-COOH$ or ester of formula $-(X_7)_{n7}-COO-X_8$, where X_6 and X_7 are selected from the group consisting of a bond, methylene, ethylene, and propylene, and where X_8 is selected from the group consisting of methyl and ethyl, and where n6 and n7 are independently 0 or 1; (viii) an amide of formula $-NHCOX_{13}$, or of formula $-CONX_{15}X_{16}$, where X_{13}, X_{15}, and X_{16} are each independently selected from the group consisting of hydrogen, methyl, and phenyl; (ix) $-SO_2NX_{18}X_{19}$, where X_{18} and X_{19} are independently selected from the group consisting of hydrogen, methyl, and ethyl; (x) an alcohol of formula $-(X_9)_{n9}-OH$ or an alkoxyalkyl moiety of formula $-(X_{10})_{n10}-O-X_{11}$, where X_9, and X_{10} are independently selected form the group consisting of methylene, ethylene, and propylene, and where X_{11} is independently selected from the group consisting of methyl, ethyl, and propyl, and where n9 and n10 are independently 0 or 1; (xi) a sulfone of formula $-SO_2-X_{22}$, where X_{22} is hydroxide; and (xi) a thioether of formula $-S-X_{25}$, where X_{25} is phenyl.

Preferably, R_6 and R_7 groups of the indolinone compounds of the invention are independently selected from the group consisting of (i) hydrogen; (ii) methyl, ethyl, propyl, and butyl groups optionally substituted with halogen, trihalomethyl, cyano, and nitro moieties; (iii) an amine of formula $-(X_1)_{n1}-NX_2X_3$, where X_2 and X_3 are independently selected from the group consisting of hydrogen and substituted saturated alkyl, and X_1 is an optionally substituted saturated alkylene, and where n1 is 0 or 1; (iv) a halogen or trihalomethyl; (v) an alcohol of formula $-(X_9)_{n9}-OH$ or an alkoxyalkyl moiety of formula $-(X_{10})_{n10}-O-X_{11}$, where X_9, and X_{10} are independently selected form the group consisting of methylene, ethylene, and propylene, and where X_{11} is independently selected form the group consisting of methyl, ethyl, and propyl, and where n9 and n10 are independently 0 or 1.

More preferably, R_6 and R_7 are independently selected from the group consisting of (i) hydrogen; (ii) methyl and ethyl; (iii) an amine of formula $-(X_1)_{n1}-NX_2X_3$, where X_2 and X_3 are independently selected from the group consisting of hydrogen, methyl, and

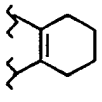
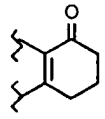
ethyl, and X_1 is selected from the group consisting of methylene and ethylene, and where n1 is 0 or 1; (iv) a halogen; (v) a hydroxy -OH or an alkoxy moiety of formula -O-X_{11}, where X_{11} is independently selected form the group consisting of methyl, ethyl, and propyl.

Preferably, the Y ring of the indolinone compound of the invention is a six-membered aromatic or heteroaromatic ring. If Y is an aromatic ring, then the moiety set forth in formula XII would take the form set forth in formula XII-A



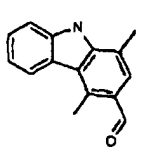
(XII-A)

with the rings optionally substituted as described herein and E having the limitations set forth herein. If Y is a heteroaromatic ring, then at least one of carbon atoms of the Y ring of the structure set forth in formula V is a heteroatom (*e.g.* nitrogen).

In some other preferred embodiments, the Y ring of the indolinone compounds is a six-membered aliphatic or heteroaliphatic ring. Then, the Y ring in the structure set forth in formula XII takes the form of, for example, optionally substituted  or  .

In other preferred embodiments, G, J, and L are independently nitrogen. X may also preferably be oxygen, nitrogen, optionally substituted with an alkyl, or be selected from the group consisting of sulfur, SO, and SO_2.

In preferred embodiments, the precursor to the T moiety of the indolinone compound of the invention set forth in formula XI is selected from the group consisting of type T aldehydes. By "type T aldehyde" is meant aldehyde compounds which are selected from the list of aldehydes numbered A-1 through A-95, and are depicted as follows.



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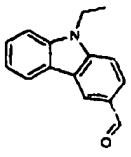

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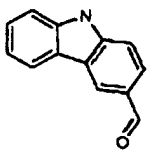

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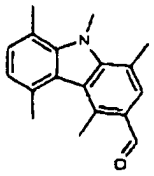
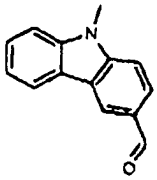
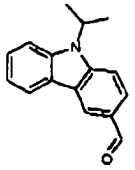
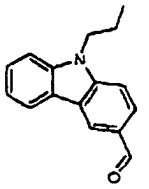
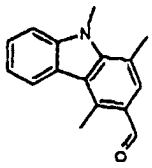
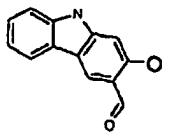
(A-4) 

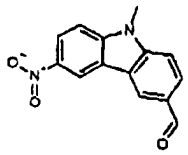
(A-5) 

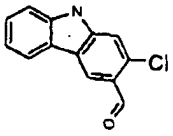
(A-6) 

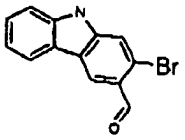
(A-7) 

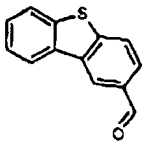
(A-8) 

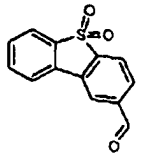
(A-9) 

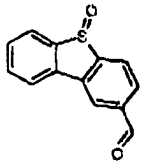
(A-10) 

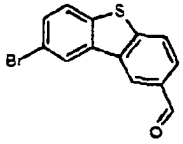
(A-11) 

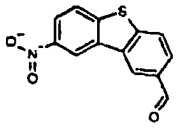
(A-12) 

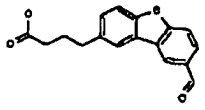
(A-13) 

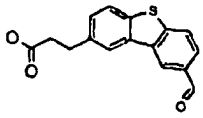
(A-14) 

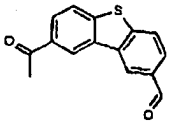
(A-15) 

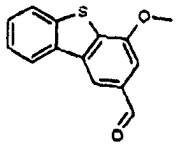
(A-16) 

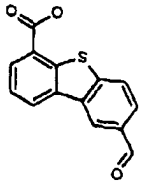
(A-17) 

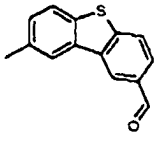
(A-18) 

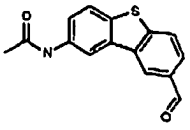
(A-19) 

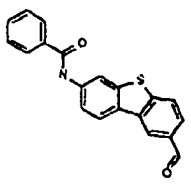
(A-20) 

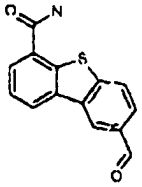
(A-21) 

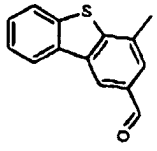
(A-22) 

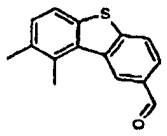
(A-23) 

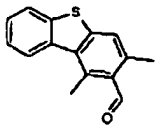
(A-24) 

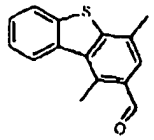
(A-25) 

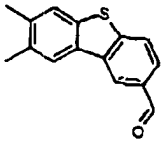
(A-26) 

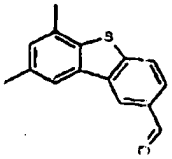
(A-27) 

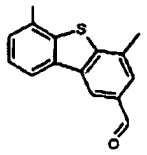
(A-28) 

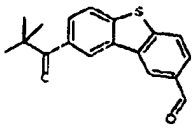
(A-29) 

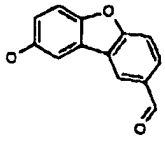
(A-30) 

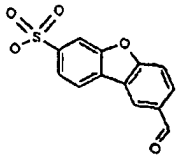
(A-31) 

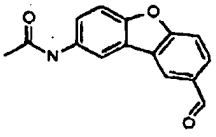
(A-32) 

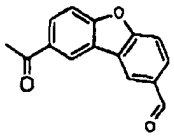
(A-33) 

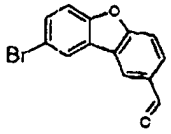
(A-34) 

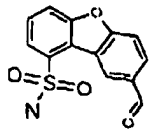
(A-35) 

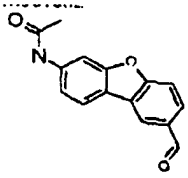
(A-36) 

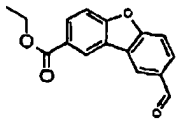
(A-37) 

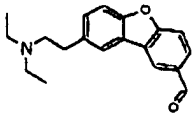
(A-38) 

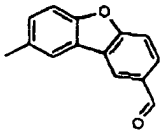
(A-39) 

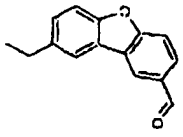
(A-40) 

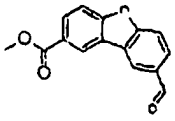
(A-41) 

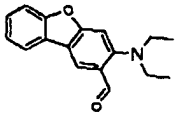
(A-42) 

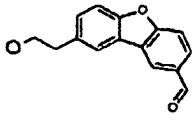
(A-43) 

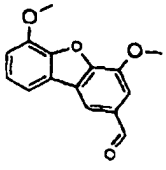
(A-44) 

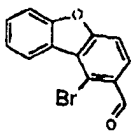
(A-45) 

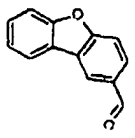
(A-46) 

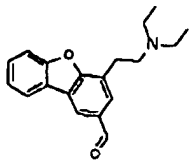
(A-47) 

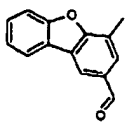
(A-48) 

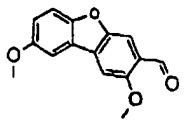
(A-49) 

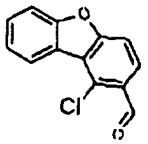
(A-50) 

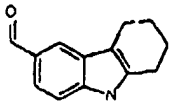
(A-51) 

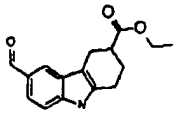
(A-52) 

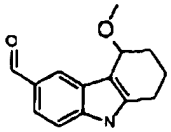
(A-53) 

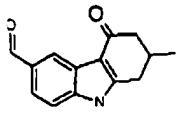
(A-54) 

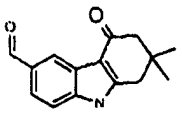
(A-55) 

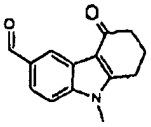
(A-56) 

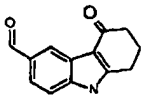
(A-57) 

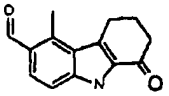
(A-58) 

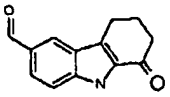
(A-59) 

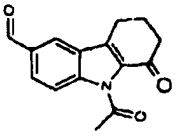
(A-60) 

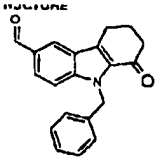
(A-61) 

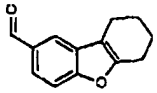
(A-62) 

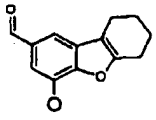
(A-63) 

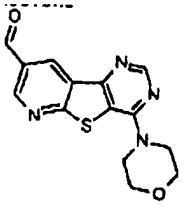
(A-64) 

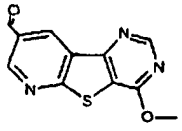
(A-65) 

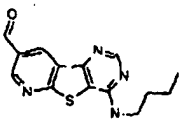
(A-66) 

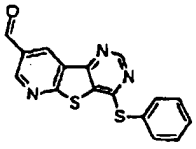
(A-67) 

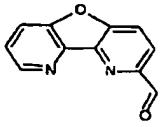
(A-68) 

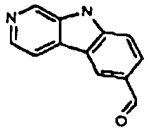
(A-69) 

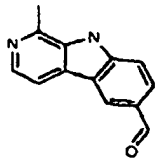
(A-70) 

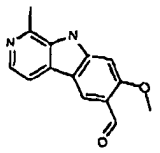
(A-71) 

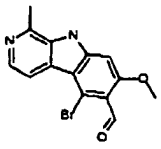
(A-72) 

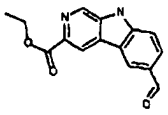
(A-73) 

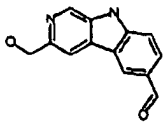
(A-74) 

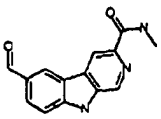
(A-75) 

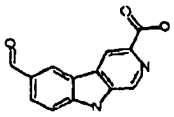
(A-76) 

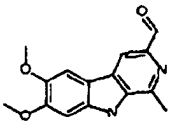
(A-77) 

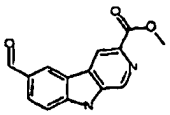
(A-78) 

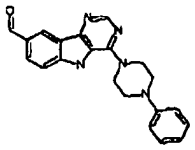
(A-79) 

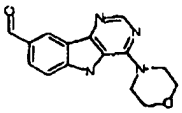
(A-80) 

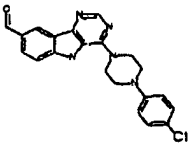
(A-81) 

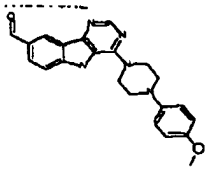
(A-82) 

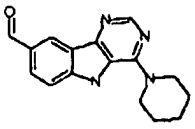
(A-83) 

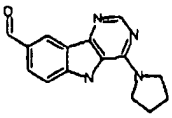
(A-84) 

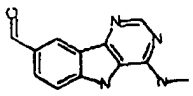
(A-85) 

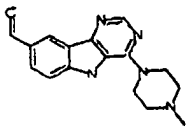
(A-86) 

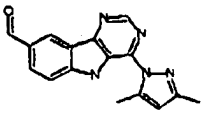
(A-87) 

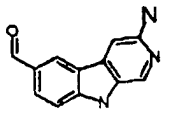
(A-88) 

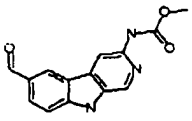
(A-89) 

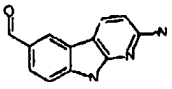
(A-90) 

(A-91) 

(A-92) 

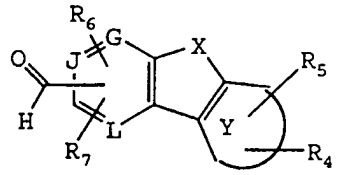
(A-93) 

(A-94) 

(A-95) 

In another aspect, the invention relates to a combinatorial library of at least 10 indolinone compounds that can be formed by reacting oxindoles with aldehydes. In preferred embodiments, the oxindoles are those that have a structure set forth in formula XIII as defined herein or any of the subgroups thereof set forth herein. The oxindoles are preferably selected from the group consisting of type Q oxindoles.

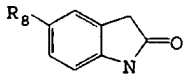
The aldehydes of the combinatorial library of the invention preferably have a structure set forth in formula XIV:



(XIV)

with R_4, R_5, R_6, R_7, G, J, L, X, and Y are as defined herein for the compound of formula XII, or any of the subgroups thereof set forth herein. Preferably, the aldehydes are selected from the group consisting of type T aldehydes.

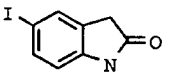
The present invention also features novel oxindole compounds. In one aspect, the invention features an oxindole compound of formula XV

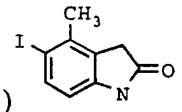


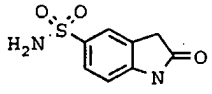
(XV)

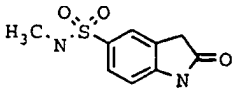
where R_8 is selected from the group consisting of (i) saturated alkyl, optionally substituted with a substituent selected from the group consisting of alkoxy, trihalomethyl, nitro, and cyano moieties, provided that the alkyl is not methyl; (ii) an amine; (iii) an iodine; (iv) a ketone of formula $-(X_4)_{n4}-CO-X_5$, where X_4 and X_5 are independently alkyl and where n4 is 0 or 1; (v) a carboxylic acid or ester; (vi) an amide; and (vii) a sulfonamide.

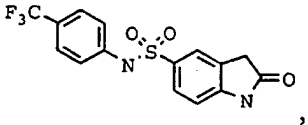
The oxindole compound of formula XV is preferably selected from the group consisting of

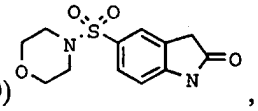
(O-11)  ,

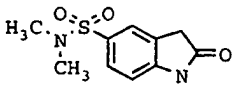
(O-31)  ,

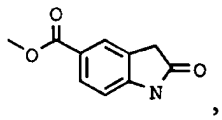
(O-17)  ,

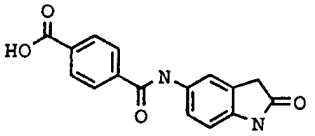
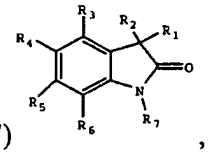
(O-18)  ,

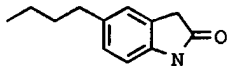
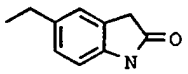
(O-19)  ,

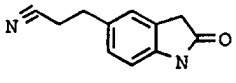
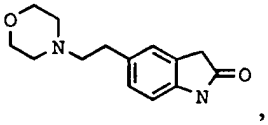
(O-20)  ,

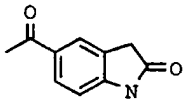
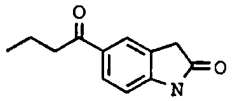
(O-45)  ,

(O-24)  ,

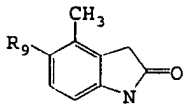
(O-36)  , (O-37)  ,

(O-33)  , (O-34)  ,

(O-55)  , (O-35)  ,

(O-53)  , and (O-54)  .

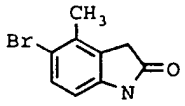
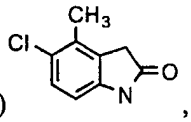
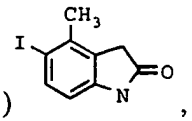
In another aspect, the invention features an oxindole compound of formula XVI

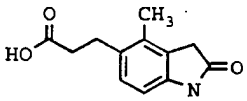
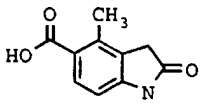
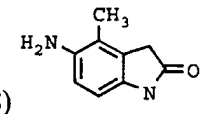


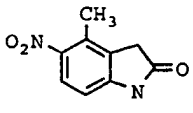
(XVI)

where R_9 is selected from the group consisting of (i) an amine; (ii) a nitro of formula - NO_2; (iii) a chlorine, bromine, or iodine; (iv) a ketone; (v) a carboxylic or ester; (vi) an amide; and (vii) a sulfonamide.

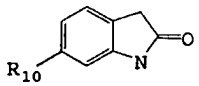
The oxindole compound of formula XVI is preferably selected from the group consisting of

(O-13)  , (O-30)  , (O-31)  ,

(O-16)  ,(O-52)  , (O-28)  ,

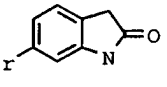
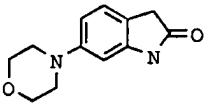
and (O-29)  .

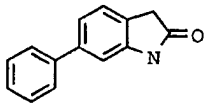
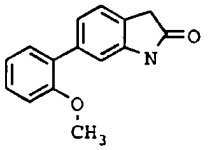
In another aspect, the invention features an oxindole compound of formula XVII

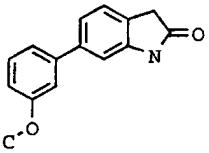
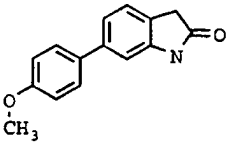
(XVII) 

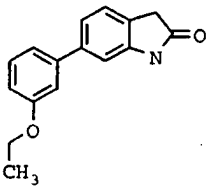
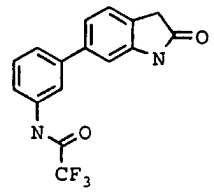
where R_{10} is selected from the group consisting of (i) an aromatic or heteroaromatic ring; (ii) an aliphatic or heteroaliphatic ring; (iii) an amine; (iv) a nitro of formula $-NO_2$; (v) a bromine; (vi) a ketone; (vii) a carboxylic acid or ester; (viii) a sulfonamide.

The oxindole compound of formula XVII is preferably selected from the group consisting of

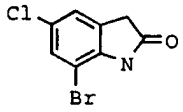
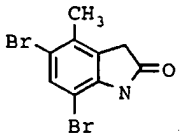
(O-51)  , (O-58)  ,

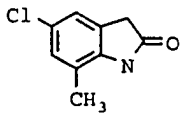
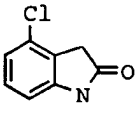
(O-46)  , (O-47)  ,

(O-48)  , (O-49)  ,

(O-50)  , and (O-60)  .

In a further aspect, the invention features an oxindole compound, where the oxindole compound is selected from the group consisting of

(O-8)  , (O-32)  ,

(O-40)  , and (O-56)  .

B. Synthesis

In a further aspect, the invention features a method for synthesizing an indolinone compound comprising the steps of:

(a) reacting a first reactant with a second reactant in a solvent and in the presence of a base at elevated temperatures, where the first reactant is an oxindole, and where the second reactant is an aldehyde;

(b) purifying the indolinone compound.

The first reactant is preferably selected from the group consisting of type Q oxindoles and the second reactant is preferably selected from the group consisting of type T aldehydes. The base is preferably a nitrogen base, and most preferably, the base is piperidine.

"Nitrogen bases" are commonly used in the art and are selected from acyclic and cyclic amines. Examples of nitrogen bases include, but are not limited to, ammonia, methyl amine, trimethylamine, aniline, and piperidine. Those skilled in the art know which nitrogen base would match the requirements of the reaction conditions.

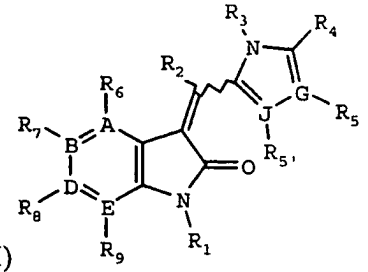
The solvent of the reaction is preferably an alcohol, and most preferably, the solvent is ethanol.

The synthetic method of the invention calls for the reaction to take place at elevated temperatures. The term "elevated temperatures" refers to temperatures that are greater than room temperature. More preferably, the elevated temperature is about 90 °C.

VI. Imidazoyl 2-Indolinone Derivatives

A. Compounds

In one aspect, the compounds of the present invention relate to imidazoyl 2-indolinone derivatives having the chemical structure of the formula XIX:



(XIX)

A, B, D and E are independently selected from the group consisting of carbon and nitrogen wherein it is understood that, when A, B, D or E is nitrogen, R_6, R_7, R_8, or R_9, respectively, does not exist and there is no bond.

G and J are selected from the group consisting of nitrogen and carbon such that, when G is nitrogen, J is carbon and when J is nitrogen, G is carbon. When either G or J is nitrogen then R_5 or $R_{5'}$, respectively, does not exist.

R_2 and the imidazolyl ring may exchange places on the double bond; *i.e.*, compound of formula XIX may exist in the E or Z configuration about the double bond at the 3-position of the 2-indolinone.

R_1 and R_3 are independently selected from the group consisting of hydrogen, alkyl, cycloalkyl, aryl, hydroxy, alkoxy, C-carboxy, O-carboxy, C-amido, C-thioamido, sulfonyl and trihalomethylsulfonyl.

R_2 is selected from the group consisting of hydrogen, alkyl, cycloalkyl, aryl, heteroaryl and halo.

R_4, R_5, and $R_{5'}$ are independently selected from the group consisting of hydrogen, alkyl, cycloalkyl, alkenyl, alkynyl, aryl, heteroaryl, heteroalicyclic, halo, trihalomethyl, hydroxy, alkoxy, aryloxy, C-carboxy, O-carboxy, carbonyl, nitro, cyano, S-sulfonamido, amino and $-NR_{10}R_{11}$.

R_{10} and R_{11} are independently selected from the group consisting of alkyl, cycloalkyl, aryl, carbonyl, sulfonyl, tri-halomethanesulfonyl and, combined, a five-member or a six-member heteroalicyclic ring.

R_6, R_7, R_8, and R_9 are independently selected from the group consisting of hydrogen, alkyl, trihaloalkyl, cycloalkyl, alkenyl, alkynyl, aryl, heteroaryl, heteroalicyclic, hydroxy, alkoxy, aryloxy, thiohydroxy, thioalkoxy, thioaryloxy, sulfinyl, sulfonyl, S-sulfonamido, N-sulfonamido, N-trihalomethanesulfonamido, carbonyl, C-carboxy, O-carboxy, cyano, nitro, halo, cyanato, isocyanato, thiocyanato, isothiocyanato, O-carbamyl, N-carbamyl, O-thiocarbamyl, N-thiocarbamyl, C-amido, N-amido, amino and $-NR_{10}R_{11}$.

R_6 and R_7 or R_7 and R_8 or R_8 and R_9, combined, may form a five or six-membered aromatic, heteroaromatic, alicyclic or heteroalicyclic ring such as, by way of example and not limitation, a methylenedioxy or an ethylenedioxy ring.

A preferred structural feature of this invention is that R_1 is hydrogen. A, B, D, and E are carbon in preferred compounds of this invention. R_2 is also hydrogen in preferred

compounds of this invention. Likewise, R_3 is hydrogen in preferred compounds of this invention.

It is also a preferred embodiment of this invention wherein all four of the above limitations are contained in the same molecule; that is, R_1, R_2, and R_3 are hydrogen and A, B, D and E are carbon.

In further preferred embodiments of this invention, R_6, R_7, R_8 and R_9 are selected from the group consisting of hydrogen, unsubstituted lower alkyl, lower alkyl substituted with a group selected from the group consisting of halo, C-carboxy and $-NR_{10}R_{11}$; unsubstituted lower alkoxy, lower alkoxy substituted with a group selected from the group consisting of halo, C-carboxy and $-NR_{10}R_{11}$; trihalomethyl, unsubstituted alkenyl, unsubstituted alkynyl, unsubstituted aryl, aryl substituted with one or more groups independently selected from the group consisting of unsubstituted lower alkyl, lower alkyl substituted with one or more halo groups, halo, C-carboxy, unsubstituted alkoxy, amino, S-sulfonamido or $-NR_{10}R_{11}$; unsubstituted heteroalicyclic, heteroalicyclic substituted with one or more groups independently selected from the group consisting of unsubstituted lower alkyl, lower alkyl substituted with one or more halo grops, aldehyde, unsubstituted lower alkyl carbonyl, hydroxy, unsubstituted alkoxy, alkoxy substituted with one or more halo groups, C-carboxy, amino, S-sulfonamido or -NR10R11; unsubstituted aryloxy, aryloxy substituted with one or more groups independently selected from the group consisting of unsubstituted lower alkyl, trihalomethyl, halo, hydroxy, amino, sulfonamido or $NR_{10}R_{11}$; thiohydroxy, unsubstituted thioalkoxy, unsubstituted thioaryloxy, thioaryloxy substituted with one or more groups independently selected from the group consisting of halo, hydroxy, amino, S-sulfonamido or $NR_{10}R_{11}$, S-sulfonamido, C-carboxy, O-carboxy, hydroxy, cyano, nitro, halo, C-amido, N-amido, amino and $-NR_{10}R_{11}$.

With regard to R_{10} and R_{11}, in preferred embodiments of this invention, one of them is hydrogen while the other is an unsubstituted lower alkyl group.

Finally, with regard to R_4, R_5 and $R_{5'}$, they are preferrably independently selected from the group consisting of hydrogen, unsubstituted lower alkyl, trihalomethyl, lower alkyl substituted on the carbon furthest from the point of attachment to the ring with a C-carboxy group, halo, hydroxy, unsubstituted alkoxy, O-carboxy, C-carboxy, amino, C-amido, N-amido, S-sulfonamido, nitro, amino and $-NR_{10}R_{11}$.

The chemical formulae referred to herein may exhibit the phenomena of tautomerism and structural isomerism. For example, the compounds described herein may adopt and E or a Z configuration about the double bond connecting the 2-indolinone moiety to the imidazoyl moiety or they may be a mixture of E and Z. The formulae shown herein are drawn with wavy lines on the imidazoyl side of the double bond to signify that the positions of R_2 and the imidazoyl group are interchangeable. This invention encompasses any tautomeric or structural isomeric form and mixtures thereof which possess the ability to modulate RTK, CTK and/or STK activity and is not limited to any one tautomeric or structural isomeric form.

B. Synthesis

In another aspect, the invention provides for a method for synthesizing an imidazoyl-2-indolinone comprising the steps of:

(a) binding a carbonylimidazole to a solid substrate;

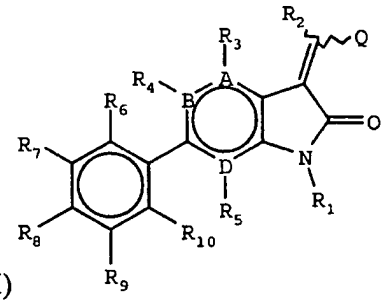
(b) reacting said solid substrate-bound carbonylimidazole with a 2-indolinone to form a solid substrate-bound imidazoyl-2-indolinone; and,

(c) releasing the imidazoly-2-indolinone from the solid substrate.

In a preferred embodiment, the solid substrate comprises a resin. More preferably, the resin comprises a 2-chlorotrityl resin. In other preferred embodiments, the method of synthesis comprises washing the solid substrate-bound imidazoyl-2-indolinone with acetone, water, dichloromethane and methanol.

VII. Phenyl 2-Indolinone Derivatives

In another aspect, the compounds of the present invention relate to phenyl 2-indolinone derivatives having the chemical structure of formula XX:



(XX)

A, B and D are independently selected from the group consisting of carbon and nitrogen wherein it is understood that, when A, B or D is nitrogen, R_3, R_4 or R_5, respectively, does not exist.

R_1 is selected from the group consisting of hydrogen, alkyl, cycloalkyl, aryl, heteroaryl, hydroxy, alkoxy, C-carboxy, O-carboxy, C-amido, C-thioamido, sulfonyl and trihalomethylsulfonyl.

R_2 is selected from the group consisting of hydrogen, alkyl, cycloalkyl, aryl, and heteroaryl.

R_3, R_4, R_5, R_6, R_7, R_8, R_9 and R_{10} are independently selected from the group consisting of hydrogen, alkyl, trihalomethyl, cycloalkyl, alkenyl, alkynyl, aryl, heteroaryl, heteroalicyclic, hydroxy, alkoxy, aryloxy, thiohydroxy, thioalkoxy, thioaryloxy, sulfinyl, sulfonyl, S-sulfonamido, N-sulfonamido, N-trihalomethanesulfonamido, carbonyl, C-carboxy, O-carboxy, cyano, azido, nitro, halo, cyanato, isocyanato, thiocyanato, isothiocyanato, O-carbamyl, N-carbamyl, O-thiocarbamyl, N-thiocarbamyl, C-amido, N-amido, amino and $-NR_{11}R_{12}$.

R_3 and R_4 or R_6 and R_7 or R_7 and R_8 or R_8 and R_9 or R_9 and R_{10} may combine to form a methylenedioxy or an ethylenedioxy group.

Q is selected from the group consisisting of aryl, heteroaryl and fused heteroaryl:cycloalkyl/heteroalicyclic.

R_{11} and R_{12} are independently selected from the group consisting of hydrogen, alkyl, cycloalkyl, aryl, carbonyl, acetyl, sulfonyl, trihalomethanesulfonyl and, combined, a five-member or a six-member heteroalicyclic ring.

A preferred embodiment of this invention is a compound in which R_1 and R_2 are hydrogen.

A further preferred embodiment of this invention is a compound in which A, B and D are carbon.

It is preferred that the above five structural limitations are incorporated into the same molecule; i.e., that, in a compound of this invention, R_1 and R_2 are hydrogen and A, B and D are carbon.

It is also a preferred embodiment of this invention that R_3, R_4 and R_5 are hydrogen.

It is likewise a preferred embodiment of this invention that R_6, R_7, R_8, R_9 and R_{10} are independently selected from the group consisting of hydrogen and unsubstituted lower alkoxy.

It is a preferred embodiment of this invention that at least one of R_6, R_7, R_8, R_9 or R_{10} is unsubstituted lower alkoxy.

Another preferred embodiment of this invention is that, when Q is aryl, the aryl group is substituted with one or more groups independently selected from the groups consisting of hydrogen, unsubstituted lower alkyl, unsubstituted lower alkoxy and heteroalicylic, in particular, 4-formylpiperazin-1-yl.

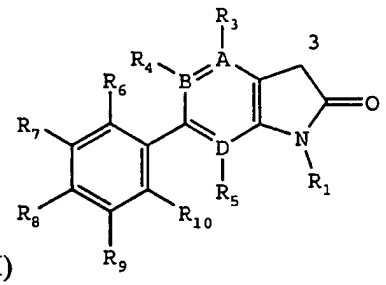
It is a preferred embodiment of this invention that when Q is heteroaryl, the heteroaryl group is selected from the group consisting of pyrrol-2-yl, imidazo-4-yl and thiophen-2-yl.

When Q is a fused heteroaryl:cycloalkyl/heteroalicyclic group, the heteroaryl moiety is preferably selected from pyrrolo, thiopheno, furano, thiazolo, oxazolo, pyridino and imidazolo. In a presently particularly preferred embodiment, Q is 4,5,6,7-tetrahydroindol-2-yl.

Finally, Q is preferably substituted with one or more groups independently selected from the group consisting of hydrogen, unsubstituted lower alkyl, unsubstituted lower alkoxy, carboxy, carboxy salt, carboxyalkyl and carboxyalkyl salt where, in the carboxyalkyl or carboxyalkyl salt, r is 1 or 2.

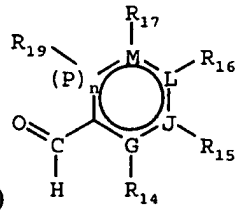
Representative compounds of this invention are shown in Table 9. The compounds shown are presented by way of example only and are not to be construed as limiting the scope of this invention in any manner whatsoever.

Another aspect of this invention is a combinatorial library of at least ten (10) compounds formed by reacting an oxindole having the general chemical structure of formula XXI:



(XXI)

with an aldehyde having the general chemical structure of formula XXII:



(XXII)

R_1, R_3, R_4, R_5, R_6, R_7, R_8, R_9 and R_{10} are as previously defined above.

G, J, L, M and P are independently selected from the group consisting of carbon and nitrogen, n is 0 or 1 and it is understood that the 5- or 6-member ring formed when any of G, J, L, M and/or P is nitrogen is one known in the chemical arts.

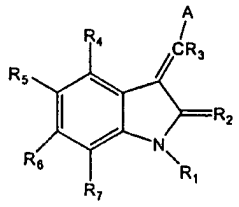
R_{14}, R_{15}, R_{16}, R_{17} and R_{18} are independently selected from the group consisting of hydrogen, alkyl, trihalomethyl, cycloalkyl, alkenyl, alkynyl, aryl, heteroaryl, heteroalicyclic, hydroxy, alkoxy, aryloxy, thiohydroxy, thioalkoxy, thioaryloxy, sulfinyl, sulfonyl, S-sulfonamido, N-sulfonamido, N-trihalomethanesulfonamido, carbonyl, C-carboxy, O-carboxy, cyano, azido, nitro, halo, cyanato, isocyanato, thiocyanato, isothiocyanato, O-carbamyl, N-carbamyl, O-thiocarbamyl, N-thiocarbamyl, C-amido, N-amido, amino and $-NR_{11}R_{12}$.

R_{14} and R_{15} or R_{15} and R_{16} or R_{16} and R_{17} or R_{17} and R_{18} may combine to form a 5- or 6-menber cycloalkyl or heteroalicyclic ring.

When G, J, L, M or P is nitrogen, R_{14}, R_{15}, R_{16}, R_{17} or R_{18}, respectively, does not exist.

VIII. Method of Modulating Cell Proliferation

Another aspect of the invention features a method of modulating cell proliferation, comprising administering to a patient in need of such treatment a pharmaceutically acceptable composition comprising a therapeutically effective amount of one or more indolinone compounds of formula XVIII:



(XVIII)

R_1 is H or alkyl; R_2 is O or S; R_3 is H;

R_4, R_5, R_6, and R_7 are each independently selected from the group consisting of hydrogen alkyl, alkoxy, aryl, aryloxy, alkaryl, alkaryloxy, halogen, trihalomethyl, S(O)R, SO_2NRR', SO_3R, SR, NO_2, NRR', OH, CN, C(O)R, OC(O)R, NHC(O)R, $(CH_2)_nCO_2R$, CONRR', and $(CH_2)_nONRR'$;

A is selected from the group consisting of a 4,5,6,7-tetrahydroindole and a five-membered heteroaryl ring, where the five-membered ring is selected from the group consisting of thiophene, pyrrole, pyrazole, imidazole, 1,2,3-triazole, 1,2,4-triazole, oxazole, isoxazole, thiazole, isothiazole, 2-sulfonylfuran, 4-alkylfuran, 1,2,3-oxadiazole, 1,2,4-oxadiazole, 1,2,5-oxadiazole, 1,3,4-oxadiazole, 1,2,3,4,-oxatriazole, 1,2,3,5-oxatriazole, 1,2,3-thiadiazole, 1,2,4-thiadiazole, 1,2,5-thiadiazole, 1,3,4-thiadiazole, 1,2,3,4-thiatriazole, 1,2,3,5-thiatriazole, and tetrazole, where the five-membered ring and the tetrahydroindole are optionally substituted with one or more substituents selected from the group consisting of alkyl, alkoxy, aryl, aryloxy, alkaryl, alkaryloxy, halogen, trihalomethyl, S(O)R, SO_2NRR', SO_3R, SR, NO_2, NRR', OH, CN, C(O)R, OC(O)R, NHC(O)R, $(CH_2)_nCO_2R$, CONRR', and $(CH_2)_nONRR'$;

n is 0-3; R is selected from the group consisting of H, alkyl, and aryl; and R' is selected from the group consisting of H, alkyl, and aryl, where the alkyl is optionally substituted with a six-membered heteroaliphatic ring, and where the six-membered ring is optionally substituted at one or more positions with substituents selected from the group consisting of alkyl, alkoxy, halogen, trihalomethyl, NO_2, and $(CH_2)_nCO_2R$.

The term "abnormal cell proliferation" or "cell proliferative disorder" as used herein refers to a disorder where an excess cell proliferation of one or more subset of cells in a multicellular organism occurs resulting in harm (*e.g.*, discomfort or decreased life expectancy) to the multicellular organism. The excess cell proliferation can be determined by reference to the general population and/or by reference to a particular patient (*e.g.*, at an earlier point in the patient's life). Hyper-proliferative cell disorders can occur in different types of animals and in humans, and produce different physical manifestations depending upon the affected cells. Hyper-proliferative cell disorders include cancer and metastases, autoimmune disorders, rheumatoid arthritis, endometriosis, ocular disease, arterial thickening and restenosis, inflammatory disorders such as psoriasis, and fibrotic disorders, such as aberrant wound healing.

In reference to the treatment of abnormal cell proliferative conditions, a therapeutic effect refers to one or more of the following: (a) a reduction in hyperproliferation; (b) inhibition of hyperproliferation; (c) inhibition (*i.e.*, slowing or stopping) of tumor metastasis; and (d) relieving to some extent one or more of the symptoms associated with the abnormal condition.

The term "signal transduction" as used herein refers to a fundamental mechanism whereby external stimuli that regulate diverse cellular processes are relayed to the interior of cells. One of the key biochemical mechanisms of signal transduction involves the reversible phosphorylation of proteins by kinases, which enables regulation of the activity of mature proteins by altering their structure and function. The protein kinases involved in signal transduction include tyrosine kinases which phosphorylate proteins on the alcohol moiety of tyrosine residues, and serine/threonine kinases which phosphorylate on serine/threonine residues.

Further, the term "signal transduction pathway" refers to the molecules that propagate an extracellular signal through the cell membrane to become intracellular signals. These signals can then stimulate a cellular response. The polypeptide molecules involved in signal transduction processes are typically receptor and non-receptor protein kinases, receptor and non-receptor protein phosphatases, adaptor molecules, nucleotide exchange factors, and transcription factors.

The term "solvent" as used herein refers to a chemical compound that facilitates the solubilization of compounds. Examples of solvents include, but are not limited to, pharmaceutically acceptable alcohols, such as ethanol; polyoxyhydrocarbyl compounds, such as poly(ethylene glycol); pharmaceutically acceptable surfactants such as CREMOPHOR EL®; polyglycolized lipids, such as GELUCIRE® and LABRASOL®; and pharmaceutically acceptable oils, such as miglyol 812.

The term "pharmaceutically acceptable alcohol" as used herein refers to alcohols which are liquids at about room temperature (approximately 20 °C). These include propylene glycol, ethanol, 2-(2-ethoxyethoxy)ethanol (TRANSCUTOL®, Gattefosse, Westwood, NJ 07675), and glycerol.

The term "polyoxyhydrocarbyl compound" as used herein refers to a water soluble carbohydrate such as glucose, sucrose, maltotriose, and the like; water soluble carbohydrate derivatives such as gluconic acid and mannitol, and oligosaccharides; and

water soluble polymers such as polyvinylpyrrolidone, poly(vinyl alcohol), and in particular, polyethers such as other polyoxyalkylenes including poly(ethylene glycol) or other water soluble mixed oxyalkylene polymers and the polymeric form of ethylene glycol. Although polyoxyhydrocarbyl compounds preferably contain more than one carbon, oxygen, and hydrogen atom, some molecules such as poly(ethylene imine) are also included.

A particularly preferred class of solubilizing polyoxyhydrocarbyl moieties comprises poly(ethylene glycol) (PEG) and PEG derivatives, such as PEG monomethyl ether. Other suitable PEG derivatives include PEG-silicon derived ethers. Many of these polymers are commercially available in a variety of molecular weights. Others may be conveniently prepared from commercially available materials, such as by coupling of amino-PEG moiety to a haloalkyl silyl or silane moiety.

Suitable PEGs may vary in molecular weight from about 200 g/mol to about 20,000 g/mol or more, more preferably 200 g/mol to 5,000 g/mol, even more preferably 250 g/mol to 1,000 g/mol, and most preferably 250 g/mol to 500 g/mol. The choice of a particular molecular weight may depend on the particular indolinone compound chosen and its molecular weight and degree of hydrophobicity, as well as the particular application for which the formulation is to be used.

The term "pharmaceutically acceptable surfactant" as used herein refers to a compound that can solubilize or aid in solubilization of indolinone compounds into aqueous solutions. Preferably for parenteral formulations, the surfactant is a non-ionic surfactant. Examples of pharmaceutically acceptable surfactants include POLYSORBATE 80® and other polyoxyethylene sorbitan fatty acid esters, glyceryl monooleate, polyvinyl alcohol, ethylene oxide copolymers such as PLURONIC® (a polyether) and TETRONIC® (BASF), polyol moieties, and sorbitan esters. Preferably ethoxylated castor oils, such as CREMOPHOR® EL, are used for the formulation of indolinone compounds.

The term "ethoxylated castor oil" as used herein refers to castor oil that is modified with at least one oxygen containing moiety. In particular the term refers to castor oil comprising at least one ethoxyl moiety.

Further, the term "pharmaceutically acceptable surfactant" as used herein in reference to oral formulations, includes pharmaceutically acceptable non-ionic surfactants

(for example polyoxyethylenepolypropylene glycol, such as POLOXAMER® 68 (BASF Corp.) or a mono fatty acid ester of polyoxyethylene (20) sorbitan monooleate (TWEEN® 80), polyoxyethylene (20) sorbitan monostearate (TWEEN® 60), polyoxyethylene (20) sorbitan monopalmitate (TWEEN® 40), polyoxyethylene (20) sorbitan monolaurate (TWEEN® 20) and the like); polyoxyethylene castor oil derivatives (for example, polyoxyethyleneglycerol-triricinoleate or polyoxyl 35 castor oil (CREMOPHOR® EL, BASF Corp.), polyoxyethyleneglycerol oxystearate (CREMOPHOR® RH 40 (polyethyleneglycol 40 hydrogenated castor oil) or CREMOPHOR® RH 60 (polyethyleneglycol 60 hydrogenated castor oil), BASF Corp.) and the like); or a pharmaceutically acceptable anionic surfactant.

The term "polyglycolized lipids" as used herein refers to mixtures of monoglycerides, diglycerides, or triglycerides and polyethyleneglycol monoesters and diesters formed by the partial alcoholysis of vegetable oil using PEG of 200 g/mol to 2,000 g/mol or by the esterification of fatty acids using PEG 200 g/mol to 2,000 g/mol and glycerols. Preferably these include GELUCIRE® 35/10, GELUCIRE® 44/14, GELUCIRE® 46/07, GELUCIRE® 50/13, GELUCIRE® 53/10, and LABRASOL®.

The term "pharmaceutically acceptable oils" as used herein refers to oils such as mineral oil or vegetable oil (including safflower oil, peanut oil, and olive oil), fractionated coconut oil, propylene glycol monolaurate, mixed triglycerides with caprylic acid and capric acid, and the like. Preferred embodiments of the invention feature mineral oil, vegetable oil, fractionated coconut oil, mixed triglycerides with caprylic acid, and capric acid. A highly preferred embodiment of the invention features Miglyol 812 (available from Huls America, USA).

In preferred embodiments of the methods of modulating abnormal cell proliferation, the composition consists of compounds of formula XVIII, where A is selected from the group consisting of pyrrole, thiophene, and 4,5,6,7-tetrahydroindole, optionally substituted with one or more substituents selected from the group consisting of alkyl, alkoxy, aryl, aryloxy, alkaryl, alkaryloxy, halogen, trihalomethyl, S(O)R, SO_2NRR', SO_3R, SR, NO_2, NRR', OH CN, C(O)R, OC(O)R, NHC(O)R, $(CH_2)_nCO_2R$, CONRR', and $(CH_2)_nONRR$; n is 0-3; R is selected from the group consisting of H, alkyl, and aryl; and R' is selected from the group consisting of H, alkyl, and aryl, wherein the alkyl is optionally substituted with a six-membered heteroaliphatic ring, and where the six-

membered ring is optionally substituted at one or more positions with substituents selected from the group consisting of alkyl, alkoxy, halogen, trihalomethyl, NO_2, and $(CH_2)_nCO_2R$. In particularly preferred embodiments, A is pyrrole. In other preferred embodiments, indolinone compounds of Formula XVIII are preferably selected from the group consisting of Compound AV-002, Compound AV-003, Compound AV-004 , Compound AV-005, Compound AV-006, Compound AV-007, and Compound AV-008.

By "Compound AV-002" is meant either the *E* or *Z* isomer of the indolinone compound, 5-amino-3-(3,5-diethyl-1H-pyrrol-2-ylmethylene)-1,3-dihydro-indol-2-one.

By "Compound AV-003" is meant either the *E* or *Z* isomer of the indolinone compound, 4-methyl-3-(3-methyl-thiophen-2-ylmethylene)-1,3-dihydro-indol-2-one.

By "Compound AV-004" is meant either the *E* or *Z* isomer of the indolinone compound, 5-chloro-3-(3,5-dimethyl-1H-pyrrol-2-ylmethylene)-1,3-dihydro-indol-2-one.

By "Compound AV-005" is meant either the *E* or *Z* isomer of the indolinone compound, 3-[4-methyl-5-(2-oxo-1,2-dihydro-indol-3-ylidenemethyl)-H-pyrrol-3-yl]-propionic acid.

By "Compound AV-006" is meant the indolinone compound, 3-[2,4-dimethyl-5-(2-oxo-1,2-dihydro-indol-3-ylidenemethyl)-1H-pyrrol-3-yl]-propionic acid.

By "Compound AV-007" is meant either the *E* or *Z* isomer of the indolinone compound, N-(2-Morpholin-4-yl-ethyl)-3-[2-(2-oxo-1,2-dihydro-indol-3-ylidenemethyl)-4,5,6,7-tetrahydro-1H-indol-3-yl]-propionamide.

By "Compound AV-008" is meant either the *E* or *Z* isomer of the indolinone compound, 3-[2-(2-Oxo-1,2-dihydro-indol-3-ylidenemethyl)-4,5,6,7-tetrahydro-1H-indol-3-yl]-propionic acid.

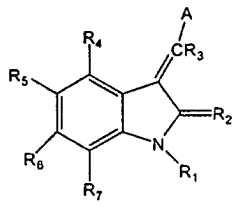
By the term "*E* or *Z* isomer" as used herein refers to the positioning of the two atoms or groups for each doubly-bonded carbon atom compared with the positioning of the two atoms or groups for the other doubly-bonded carbon. The priority of the two atoms or groups is identified for each doubly-bonded carbon atom, and then the positioning relative to each other is determined, *i.e.* whether the higher priority atoms or groups for each doubly-bonded carbon atom are on the same or opposite sides. By *Z* is meant on the same side, and by *E*, on the opposite side. "Priority" refers to atomic number, with the atom of higher atomic weight getting the higher priority. If two atoms are isotopes of the same element, the atom of higher mass number has the higher priority.

If the relative priority of the two groups attached to the doubly-bonded carbon cannot be decided, a similar comparison is made with the atoms attached to these groups, and so on.

In other preferred embodiments of the methods of modulating abnormal cell proliferation, the composition further comprises one or more pharmaceutically acceptable excipients in a formulation, where the formulation is selected from the group consisting of, but not limited to, a parenteral, a topical and an oral formulation. The effective amount of the compound in the composition comprises 1 to 1000 mg/m^2/day, preferably 10 to 500 mg/m^2/day, and most preferably 10 to 250 mg/m^2/day of one or more indolinone compounds. Preferably the patient is a mammal; more preferably the mammal is a human. Alternatively, the mammal can be a rat, in which case altered activity of VEGF, FGF, and/or PDGF can be induced.

The term "mammal" as used herein preferably refers to such organisms as mice, rats, rabbits, guinea pigs, goats, sheep, horses, and cows, for example; more preferably to dogs, cats, monkeys, and apes; and most preferably to humans.

Another aspect of the invention features methods of modulating the activity of VEGF, FGF, and/or PDGF on cells *in vitro*, or more preferably, *in vivo*, comprising administering to said cells a pharmaceutically acceptable composition comprising a therapeutic amount of one or more indolinone compounds of formula XVIII:



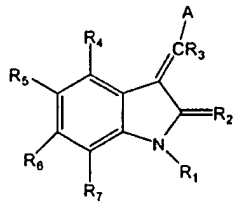
(XVIII)

as defined herein or any of the subgroups thereof set forth herein.

In preferred embodiments of the methods of modulating the activity of VEGF, FGF, or PDGF on cells, the composition further comprises one or more pharmaceutically acceptable excipients in the formulation. Preferably, the formulation is selected from the group consisting of a parenteral, an oral, and a topical formulation and the effective amount of the compound in the composition comprises 1 to 1000 mg/m^2/day, preferably 10 to 500 mg/m^2/day, and most preferably 10 to 250 mg/m^2/day of one or more indolinone compounds. Preferably the patient is a mammal; more preferably the mammal is a human.

Alternatively, the mammal can be a rat, in which case altered activity of VEGF, FGF, and/or PDGF can be induced.

The invention also features in another aspect, methods of modulating tyrosine kinase signal transduction, comprising administering to a patient in need of such treatment a pharmaceutically acceptable composition comprising a therapeutically effective amount of one or more indolinone of formula XVIII:



(XVIII)

as defined herein or any of the subgroups thereof set forth herein.

In preferred embodiments of the methods of modulating tyrosine kinase signal transduction, the composition further comprises one or more pharmaceutically acceptable excipients in the formulation. Preferably, the formulation is selected from the group consisting of a parenteral, an oral, and a topical formulation and the effective amount of the compound in the composition comprises 1 to 1000 mg/m^2/day, preferably 10 to 500 mg/m^2/day, and most preferably 10 to 250 mg/m^2/day of one or more indolinone compounds selected from the group consisting of Compound AV-002, Compound AV-003, and Compound AV-004. Preferably the patient is a mammal; more preferably the mammal is a human. Alternatively, the mammal can be a rat, in which case altered activity of VEGF, FGF, and/or PDGF can be induced.

Another aspect of the invention features methods of identifying one or more indolinone compounds that inhibit growth factor-stimulated or platelet-derived growth factor-stimulated cell proliferation comprising the following steps: (a) contacting cells with one or more indolinone compounds of formula XVIII; (b) contacting the cells with one or more growth factors selected from the group consisting of VEGF, PDGF, and FGF; and (c) monitoring an effect upon the cells. Preferably, the growth factor is VEGF and the cells are endothelial cells, or PDGF and the cells are smooth muscle cells, or FGF. Preferably, the effect is monitored colorimetrically, for example using a change in absorbance.

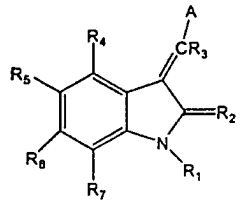
The indolinone compounds of the invention preferably modulate the activity of the protein kinase *in vitro*. These compounds preferably show positive results in one or more *in vitro* assays for an activity corresponding to treatment of the disease or disorder in question (such as the assays described in the Examples below).

The invention also features methods of identifying one or more indolinone compounds that are active in an adjuvant arthritis model in rats comprising the following steps: (a) administration of one or more indolinone compounds of formula XVIII to the rats; and (b) monitoring an effect upon the rats. Preferably, the compounds are administered at a concentration of 1 to 1000 mg/m^2/day, preferably 10 to 500 mg/m^2/day, and most preferably 10 to 250 mg/m^2/day of one or more indolinone compounds and the effect on the rats' disease is selected from the group consisting of ear nodulation, tail nodulation, nose swelling, paw swelling, and balanitis.

The term "adjuvant arthritis model" is used herein to refer to rats, preferably Wistar-Lewis or other rat strains commonly known to those skilled in the art, in which disease was induced by injecting 0.1 mL Freund's adjuvant into the base of the tail. This adjuvant arthritis model is only one example of an animal model that can be used to test the compounds of the invention. For a review of the three most common animal models, *see* Oliver & Brahn (1996) J. Rheumatol. 23:56-60, hereby enclosed herein by reference in its entirety, including any drawings, figures, or tables.

An additional aspect of the invention features methods of modulating abnormal cell proliferation, the activity of VEGF, FGF, or PDGF on cells *in vivo* or *in vitro*, or tyrosine kinase signal transduction, comprising administering to a patient in need of such treatment a pharmaceutically acceptable composition comprising a therapeutically effective amount of one or more indolinone compounds of formula XVIII identified by their ability to inhibit VEGF-, FGF-, or PDGF-stimulated cell proliferation, or inhibit one or more of the effects of adjuvant arthritis in rats. Preferably, the compounds are administered in a composition that further comprises one or more pharmaceutically acceptable excipients in a formulation that can be, but is not limited to being, administered orally, parenterally, or topically. The compounds may be administered at a concentration comprising 1 to 1000 mg/m^2/day, preferably 10 to 500 mg/m^2/day, and most preferably 10 to 250 mg/m^2/day of one or more indolinone compounds, preferably to a mammal, and more preferably to a human.

Another aspect of the invention features methods of treating or preventing an abnormal condition by administering to a patient in need of such treatment a pharmaceutically acceptable composition comprising a therapeutically effective amount of one or more indolinone compounds of formula XVIII:



(XVIII)

as defined herein or any of the subgroups thereof set forth herein.

Additional methods of treating or preventing an abnormal condition include, administering to a patient in need of such treatment a pharmaceutically acceptable composition comprising a therapeutically effective amount of one or more compounds identified by previously described methods for identifying compounds that modulate VEGF-,FGF-, or PDGF-activity, or that are active in an adjuvant arthritis model in rats, where the abnormal condition is selected from the group consisting of rheumatoid arthritis, endometriosis, ocular diseases, cancer and metastases, psoriasis, arterial thickening and restenosis, tissue ischemia, and excessive scarring during wound healing. The disease is preferably endometriosis or rheumatoid arthritis, and the composition further comprises one or more pharmaceutically acceptable excipients in a formulation that is selected from the group consisting of an intra-muscular, a depot, a parenteral, an oral, and a topical formulation. Preferably, the compounds inhibit tyrosine kinase activity *in vitro*, and the patient is a mammal, or preferably a human. Alternatively, the mammal can be a rat, in which case the stated diseases or disorders can be induced.

IX. Enhancement of Sexual Activity

In a further aspect, the invention provides for a method of increasing the sexual function of a mammal by administering to the mammal a formulation containing an indolinone compound. The invention also provides for a method of treating sexual dysfunction in a mammal in need thereof, comprising the steps of (a) administering to the mammal a formulation containing an indolinone compound; (b) monitoring the sexual activity of the mammal; and (c) adjusting the dose of the compound to match the specific

needs of the mammal. Another aspect of the invention provides for a method of preventing sexual dysfunctions in mammals, comprising the steps of (a) administering to a mammal a formulation containing an indolinone compound; (b) monitoring the sexual activity of the mammal; and (c) adjusting the dose of the compound to match the specific needs of the mammal.

"Mammal" generally refers to such organisms as mice, rats, rabbits, dogs, cats, pigs, cows, sheep, goats, more preferably monkeys and apes, and most preferably humans. Both male and female mammals are contemplated as subjects for treatment by the methods of the present invention.

"Sexual function" refers to performing any sexual acitivity, including sexual desire, intercourse, and orgasm. Thus, "sexual dysfunction" refers to any malady that would result in loss of sexual activity or the inability of the subject to perform a sexual act. The sexual dysfunctions contemplated by this invention include those dysfunctions that have an organic basis (*e.g.*, impotence) rather than a psychosexual basis.

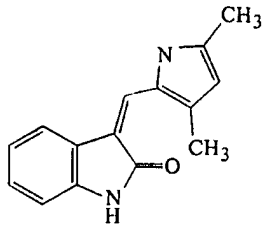
A common sexual dysfunction is impotence. "Impotence" is a sexual dysfunction that occurs in male mammals and consists of the inability of the male to achieve or maintain an erection, which is the elongation and hardening of the penis, sufficient to penetrate the vagina of a female or to perform a sexual act. Impotence does not necessarily involve lack of sexual desire. In general, impotent males have sexual desire but are not able to perform the sexual act. Therefore, impotence refers to two different conditions: one in which the male cannot obtain an erection, and the second in which, once an erection is obtained, the male cannot maintain it long enough for the completion of the sexual act. The treatment of both of these conditions is within the scope of the present invention.

Other sexual dysfunctions in mammals include loss of sexual desire and the inability to reach orgasm, and in female mammals, vaginal dryness. In male mammals, orgasm is normally coincident with ejaculation, which is the ejection of semen through the penis.

In the methods of the present invention, an indolinone compound is administered to a mammal suffering from a sexual dysfunction. "Administering" relates broadly to the provision to an organism and more specifically to a method of introducing a compound into cells or tissues of an organism. Many techniques exist in the art to administer

compounds to an organism, including (but not limited to) oral, parenteral, dermal, injection, and aerosol applications.

Preferably, the indolinone compound used in the methods of the invention is 3-[(2,4-dimethylpyrrol-5-yl)methylene]-2-indolinone, which has the structure set forth in formula XXIII.



(XXIII)

The methods of the invention also include a pharmaceutical composition comprising an indolinone compound and a pharmaceutically acceptable diluent or carrier.

Subsequent to administering an indolinone compound to a mammal, the sexual activity of the mammal may be monitored and any changes or improvements may be noted. The term "monitoring" refers to observing the effect of adding a compound of the invention to a mammal. The effect can be manifested in a change in the sexual activity of the mammal. "Sexual activity" can be, for example, the mammal's sexual desire, its ability to perform a sexual act, or its ability to have an orgasm.

In another aspect, the invention provides a method of identifying an indolinone compound which increases sexual function in a mammal, comprising the steps of (a) administering to the mammal a formulation containing the indolinone compound; (b) monitoring the sexual activity of the mammal; and (c) identifying an indolinone compound which modulates the sexual activity of the mammal. The invention also provides a method for synthesizing an indolinone compound that was identified by the methods of the invention to be a modulator of mammalian sexual activity.

The term "modulates" refers to altering the sexual activity of a mammal by, for example, increasing or decreasing the frequency of having sexual desires, obtaining an erection, or achieving orgasm. A modulator preferably increases the sexual activity of the mammal.

In a further aspect, the invention provides a method of increasing sexual function in a mammal in need of such increase comprising the steps of (a) identifying the mammal

in need of the increase in sexual function; and (b) administering to the mammal a formulation containing an indolinone compound. The identification step may be conducted by a health care professional as part of the mammal's medical diagnosis, or, if the mammal is a human, he or she may self-diagnose the need for the increase in his or her sexual function.

X. Compositions, Methods of Treatment

In another aspect, the invention features a pharmaceutical composition comprising (i) a physiologically acceptable carrier, diluent, or excipient; and (ii) an indolinone compound as described herein, or a salt thereof.

The invention also features a method of modulating the function of a protein kinase with a compound of the invention, comprising the step of contacting cells expressing the protein kinase with the compound.

The compounds of the invention preferably modulate the activity of the protein kinase in vitro. These compounds preferably show positive results in one or more in vitro assays for an activity corresponding to treatment of the disease or disorder in question (such as the assays described in the Examples below).

The invention also features a method of identifying indolinone compounds that modulate the function of protein kinase, comprising the following steps: (a) contacting cells expressing the protein kinase with the compound; and (b) monitoring an effect upon the cells. The effect upon the cells is preferably a change or an absence of a change in cell phenotype, more preferably it is a change or an absence of a change in cell proliferation, even more preferably it is a change or absence of a change in the catalytic activity of the protein kinase, and most preferably it is a change or absence of a change in the interaction between the protein kinase with a natural binding partner, as described herein.

In a preferred embodiment, the invention features a method for identifying the indolinone compounds of the invention, comprising the following steps: (a) lysing the cells to render a lysate comprising protein kinase; (b) adsorbing the protein kinase to an antibody; (c)incubating the adsorbed protein kinase with a substrate or substrates; and (d) adsorbing the substrate or substrates to a solid support or antibody; where the step of monitoring the effect on the cells comprises measuring the phosphate concentration of the substrate or substrates.

In yet another aspect, the invention features a method for treating a disease related to unregulated tyrosine kinase signal transduction, where the method includes the step of administering to a subject in need thereof a therapeutically effective amount of an indolinone compound as described herein.

The invention also features a method of regulating tyrosine kinase signal transduction comprising administering to a subject a therapeutically effective amount of a compound as described herein.

Furthermore, the invention features a method of preventing or treating an abnormal condition in an organism, where the abnormal condition is associated with an aberration in a signal transduction pathway characterized by an interaction between a protein kinase and a natural binding partner, where the method comprises the following steps: (a) administering a compound as described herein; and (b) promoting or disrupting the abnormal interaction. The organism is preferably a mammal and the abnormal condition is preferably cancer.

The summary of the invention described above is non-limiting and other features and advantages of the invention will be apparent from the following description of the preferred embodiments, and from the claims.

BRIEF DESCRIPTION OF THE DRAWINGS

Table 1 shows a summary of IC_{50} determinations of the inhibition of VEGF-stimulation by the indolinone compounds, Compound AV-002, Compound AV-003, and Compound AV-004, of human umbilical vein endothelial cells (HUVEC), in the absence and presence of exogenously applied VEGF. The effect of doxorubicin is included for comparison.

Table 2 shows a summary of IC_{50} determinations of the activity of the indolinone compounds, Compound AV-002, Compound AV-003, and Compound AV-004, for embryonic thoracic aorta smooth muscle cells (A10 cells), in the absence of exogenously applied PDGF.

Table 3 shows a summary of IC_{50} determinations of the activity of the indolinone compounds, Compound AV-002, Compound AV-003, and Compound AV-004, with pre-incubation, for A10 cells, in the absence of exogenously applied PDGF. The effect of doxorubicin is included for comparison.

Table 4 shows a summary of IC_{50} determinations of the inhibition of PDGF-stimulation by the indolinone compounds, Compound AV-002, Compound AV-003, and Compound AV-004, of A10 cells without pre-incubation.

Table 5 shows a summary of IC_{50} determinations of the inhibition of PDGF-stimulation by the indolinone compounds, Compound AV-002, Compound AV-003, and Compound AV-004, of A10 cells with pre-incubation. The effect of doxorubicin is included for comparison.

Table 6 shows a summary of the effects of test compounds (Compound AV-002, Compound AV-003, and Compound AV-004) in the adjuvant arthritis rat model compared to arthritis control (vehicle only).

Table 7 shows a summary of the effects of test compounds (Compound AV-002, Compound AV-003, and Compound AV-004) in the adjuvant arthritis rat model compared to arthritis control (vehicle only) as an arthritis index.

Figure 1 shows the effect of Compound AV-002 on paw volume of adjuvant arthritis rats.

Figure 2 shows the effect of Compound AV-003 on paw volume of adjuvant arthritis rats.

Figure 3 shows the effect of Compound AV-004 on paw volume of adjuvant arthritis rats.

Figure 4 shows the effect of test compounds on body weight of adjuvant arthritis rats.

DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to compounds capable of regulating and/or modulating protein kinase signal transduction and more particularly receptor and non-receptor protein kinase signal transduction.

Receptor tyrosine kinase mediated signal transduction is initiated by extracellular interaction with a specific growth factor (ligand), followed by receptor dimerization, transient stimulation of the intrinsic protein kinase activity and phosphorylation. Binding sites are thereby created for intracellular signal transduction molecules and lead to the formation of complexes with a spectrum of cytoplasmic signaling molecules that facilitate the appropriate cellular response (*e.g.*, cell division, metabolic effects to the extracellular

microenvironment). *See*, Schlessinger and Ullrich, 1992, *Neuron* 9:303-391.

It has been shown that tyrosine phosphorylation sites in growth factor receptors function as high-affinity binding sites for SH2 (*src* homology) domains of signaling molecules. Fantl *et al.*, 1992, *Cell 69*:413-423; Songyang *et al.*, 1994, *Mol. Cell. Biol. 14*:2777-2785); Songyang *et al.*, 1993, *Cell 72*:767-778; and Koch *et al.*, 1991, *Science 252*:668-678. Several intracellular substrate proteins that associate with receptor tyrosine kinases have been identified. They may be divided into two principal groups: (1) substrates which have a catalytic domain; and (2) substrates which lack such domain but serve as adapters and associate with catalytically active molecules. Songyang *et al.*, 1993, *Cell 72*:767-778. The specificity of the interactions between receptors and SH2 domains of their substrates is determined by the amino acid residues immediately surrounding the phosphorylated tyrosine residue. Differences in the binding affinities between SH2 domains and the amino acid sequences surrounding the phosphotyrosine residues on particular receptors are consistent with the observed differences in their substrate phosphorylation profiles. Songyang *et al.*, 1993, *Cell 72*:767-778. These observations suggest that the function of each receptor tyrosine kinase is determined not only by its pattern of expression and ligand availability but also by the array of downstream signal transduction pathways that are activated by a particular receptor. Thus, phosphorylation provides an important regulatory step which determines the selectivity of signaling pathways recruited by specific growth factor receptors, as well as differentiation factor receptors.

Tyrosine kinase signal transduction results in, among other responses, cell proliferation, differentiation and metabolism. Abnormal cell proliferation may result in a wide array of disorders and diseases, including the development of neoplasia such as carcinoma, sarcoma, leukemia, glioblastoma, hemangioma, psoriasis, arteriosclerosis, arthritis and diabetic retinopathy (or other disorders related to uncontrolled angiogenesis and/or vasculogenesis).

This invention is therefore directed to compounds which regulate, modulate and/or inhibit tyrosine kinase signal transduction by affecting the enzymatic activity of the RTKs and/or the non-receptor tyrosine kinases and interfering with the signal transduced such proteins. More particularly, the present invention is directed to compounds which regulate, modulate and/or inhibit the RTK and/or non-receptor tyrosine kinase mediated

signal transduction pathways as a therapeutic approach to cure many kinds of solid tumors, including but not limited to carcinoma, sarcoma, leukemia, erythroblastoma, glioblastoma, meningioma, astrocytoma, melanoma and myoblastoma. Indications may include, but are not limited to brain cancers, bladder cancers, ovarian cancers, gastric cancers, pancreas cancers, colon cancers, blood cancers, lung cancers and bone cancers.

I. Target Diseases to be Treated by the Compounds of the Invention

The compounds described herein are useful for treating disorders related to unregulated tyrosine kinase signal transduction, including cell proliferative disorders, fibrotic disorders and metabolic disorders.

Cell proliferative disorders which can be treated or further studied by the present invention include cancers, blood vessel proliferative disorders and mesangial cell proliferative disorders.

Blood vessel proliferative disorders refer to angiogenic and vasculogenic disorders generally resulting in abnormal proliferation of blood vessels. The formation and spreading of blood vessels, or vasculogenesis and angiogenesis, respectively, play important roles in a variety of physiological processes such as embryonic development, corpus luteum formation, wound healing and organ regeneration. They also play a pivotal role in cancer development. Other examples of blood vessel proliferation disorders include arthritis, where new capillary blood vessels invade the joint and destroy cartilage, and ocular diseases, like diabetic retinopathy, where new capillaries in the retina invade the vitreous, bleed and cause blindness. Conversely, disorders related to the shrinkage, contraction or closing of blood vessels, such as restenosis, are also implicated.

Fibrotic disorders refer to the abnormal formation of extracellular matrix. Examples of fibrotic disorders include hepatic cirrhosis and mesangial cell proliferative disorders. Hepatic cirrohis is characterized by the increase in extracellular matrix constituents resulting in the formation of a hepatic scar. Hepatic cirrhosis can cause diseases such as cirrhosis of the liver. An increased extracellular matrix resulting in a hepatic scar can also be caused by viral infection such as hepatitis. Lipocytes appear to play a major role ion hepatic cirrhosis. Other fibrotic disorders implicated include atherosclerosis (*see*, below).

Mesangial cell proliferative disorders refer to disorders brought about by abnormal

proliferation of mesangial cells. Mesangial proliferative disorders include various human renal diseases, such as glomerulonephritis, diabetic nephropathy, malignant nephrosclerosis, thrombotic microangiopathy syndromes, transplant rejection, and glomerulopathies. The PDGF-R has been implicated in the maintenance of mesangial cell proliferation. Floege *et al.*, 1993, *Kidney International* 43:47S-54S.

PKs have been associated with such cell proliferative disorders. For example, some members of the RTK family have been associated with the development of cancer. Some of these receptors, like the EGFR (Tuzi *et al.*, 1991, *Br. J. Cancer 63*:227-233; Torp *et al.*, 1992, *APMIS 100*:713-719) HER2/neu (Slamon *et al.*, 1989, *Science 244*:707-712) and the PDGF-R (Kumabe *et al.*, 1992, *Oncogene 7*:627-633) are overexpressed in many tumors and/or persistently activated by autocrine loops. In fact, in the most common and severe cancers these receptor overexpressions (Akbasak and Suner-Akbasak *et al.*, 1992, *J. Neurol. Sci. 111*:119-133; Dickson *et al.*, 1992, *Cancer Treatment Res. 61*:249-273; Korc *et al.*, 1992, *J. Clin. Invest. 90*:1352-1360) and autocrine loops (Lee and Donoghue, 1992, *J. Cell. Biol. 118*:1057-1070; Korc *et al.*, *supra*; Akbasak and Suner-Akbasak *et al.*, *supra*) have been demonstrated. For example, the EGFR receptor has been associated with squamous cell carcinoma, astrocytoma, glioblastoma, head and neck cancer, lung cancer and bladder cancer. HER2 has been associated with breast, ovarian, gastric, lung, pancreas and bladder cancer. The PDGF-R has been associated with glioblastoma, lung, ovarian, melanoma and prostate. The RTK c-met has been generally associated with hepatocarcinogenesis and thus hepatocellular carcinoma. Additionally, c-met has been linked to malignant tumor formation. More specifically, the RTK c-met has been associated with, among other cancers, colorectal, thyroid, pancreatic and gastric carcinoma, leukemia and lymphoma. Additionally, over-expression of the c-met gene has been detected in patients with Hodgkins disease, Burkitts disease, and the lymphoma cell line.

The IGF-IR, in addition to being implicated in nutritional support and in type-II diabetes, has also been associated with several types of cancers. For example, IGF-I has been implicated as an autocrine growth stimulator for several tumor types, *e.g.* human breast cancer carcinoma cells (Arteaga *et al.*, 1989, *J. Clin. Invest. 84*:1418-1423) and small lung tumor cells (Macauley *et al.*, 1990, *Cancer Res. 50*:2511-2517). In addition, IGF-I, integrally involved in the normal growth and differentiation of the nervous system,

appears to be an autocrine stimulator of human gliomas. Sandberg-Nordqvist *et al.*, 1993, *Cancer Res. 53*:2475-2478. The importance of the IGF-IR and its ligands in cell proliferation is further supported by the fact that many cell types in culture (fibroblasts, epithelial cells, smooth muscle cells, T-lymphocytes, myeloid cells, chondrocytes, osteoblasts, the stem cells of the bone marrow) are stimulated to grow by IGF-I. Goldring and Goldring, 1991, *Eukaryotic Gene Expression 1*:301-326. In a series of recent publications, Baserga even suggests that IGF-I-R plays a central role in the mechanisms of transformation and, as such, could be a preferred target for therapeutic interventions for a broad spectrum of human malignancies. Baserga, 1995, *Cancer Res. 55*:249-252; Baserga, 1994, *Cell 79*:927-930; Coppola *et al.*, 1994, *Mol. Cell. Biol. 14*:4588-4595.

The association between abnormalities in RTKs and disease are not restricted to cancer, however. For example, RTKs have been associated with metabolic diseases like psoriasis, diabetes mellitus, wound healing, inflammation, and neurodegenerative diseases. These diseases include, but are not limited to hypertension, depression, generalized anxiety disorder, phobias, post-traumatic stress syndrome, avoidant personality disorder, sexual dysfunction, eating disorders, obesity, chemical dependencies, cluster headache, migraine, pain, Alzheimer's disease, obsessive-compulsive disorder, panic disorder, memory disorders, Parkinson's disease, endocrine disorders, vasospasm, cerebellar ataxia, and gastrointestinal tract disorders. For example, the EGF-R is indicated in corneal and dermal wound healing. Defects in the Insulin-R and the IGF-1R are indicated in type-II diabetes mellitus. A more complete correlation between specific RTKs and their therapeutic indications is set forth in Plowman *et al.*, 1994, *DN&P 7*:334-339.

Not only receptor type tyrosine kinases, but also many cellular tyrosine kinases (CTKs) including src, abl, fps, yes, fyn, lyn, lck, blk, hck, fgr, yrk (reviewed by Bolen *et al.*, 1992, *FASEB J. 6*:3403-3409) are involved in the proliferative and metabolic signal transduction pathway and thus in indications of the present invention. For example, mutated src (v-src) has been demonstrated as an oncoprotein ($pp60^{v\text{-}src}$) in chicken. Moreover, its cellular homolog, the proto-oncogene $pp60^{c\text{-}src}$ transmits oncogenic signals of many receptors. For example, overexpression of EGF-R or HER2/neu in tumors leads to the constitutive activation of $pp60^{c\text{—}src}$, which is characteristic for the malignant cell but absent from the normal cell. On the other hand, mice deficient for the expression of c-src exhibit an osteopetrotic phenotype, indicating a key participation of c-src in osteoclast

function and a possible involvement in related disorders. Similarly, Zap 70 is implicated in T-cell signaling.

Furthermore, the identification of CTK modulating compounds to augment or even synergize with RTK aimed blockers is an aspect of the present invention.

Finally, both RTKs and non-receptor type kinases have been connected to hyperimmune disorders.

Alterations in the function of a protein kinase that normally regulates cell proliferation can lead to enhanced or decreased cell proliferative conditions evident in certain diseases. Aberrant cell proliferative conditions include angiogenic and vasculogenic disorders including rheumatoid arthritis, endometriosis, ocular diseases, cancer and metastases, psoriasis, arterial thickening and restenosis, tissue ischemia, and excessive scarring during wound healing.

Cancer and Metastasis

The link between angiogenesis and cancer is well established. Neovascularization is an important step in the transition from hyperplasia to neoplasia and it must occur for tumors to grow beyond 1 to 2 mm^3 (Folkman, *J. Natl. Cancer Inst.* **1990**, *82*, 4-6; Folkman, *et al.*, *Nature* **1989**, *339*, 58-61). A correlation between microvessel density and severity of disease has been observed in a number of different tumor types including malignant glioma (Plate & Risau, *GLIA* **1995**, *15*, 339-347), and breast (Horak, *et al.*, *Lancet* **1992**, *340*, 1120-1124), bladder (Dickinson, *et al., Br. J. Urol.* **1994**, *74*, 762-766), colon (Takahashi, *et al., Cancer Res.* **1995**, *55*, 3964-3968), and endometrial cancer (Kirschner, *et al.*, *Am. J. Obstet. Gynecol.* **1996**, *174*, 1879-1882).

Many activators of tumor angiogenesis are growth factors which stimulate proliferation of endothelial cells. The roles of VEGF and its cognate receptor Flk-1/KDR are well established. VEGF is secreted by a number of human tumor cell lines in culture, including glioma (Tsai, *et al., J. Neurosurg* **1995**, *82,* 864-867), melanoma (Claffey, *et al., Cancer Res.* **1996**, *56*, 172-181.) Kaposi sarcoma, and epidermoid carcinoma cells (Myoken, *et al., Proc. Natl. Acad. Sci. USA* **1991**, *88,* 5819-5823). More importantly, VEGF transcripts or protein has been identified by *in situ* hybridization or immunohistochemistry in primary gliomas (Plate, *et al., Lab Invest.* **1992**, *67,* 529-534; Plate, *et al., Int. J. Cancer* **1994**, *59,* 520-529), hemangioblastomas (Hatva, *et al., Amer. J.*

Pathol. **1996**, *148*, 763-775) and breast (Toi, *et al.*, *Jpn. J. Cancer Res.* **1994**, *85*, 1045-1049; Anan, *et al.*, *Surgery* **1996**, *119*, 333-339; Yoshiji, *et al.*, *Cancer Res.* **1996**, *56*, 2013-2016), colon (Brown, *et al.*, *Cancer Res.* **1993**, *53*, 4727-4735; Takahashi, *et al.*, *Cancer Res.* **1995**, *55*, 3964-3968) and renal cell tumors (Takahashi, *et al.*, *Cancer Res.* **1994**, *54*, 4233-4237). In glioblastoma, the message for VEGF is found in cells adjacent to necrotic regions which is consistent with upregulation by hypoxia (Shweiki, *et al.*, *Nature* **1992**, *359*, 843-845; Plate, et al, *Lab Invest.* **1992**, *67*, 529-534). Furthermore, patients with cancer have significantly higher serum VEGF levels than normal volunteers. The highest VEGF concentrations were observed in patients with untreated metastatic cancers.

A number of animal models have been developed to investigate the function of VEGF in tumor angiogenesis. Rat C6 glioma and human U87MG glioblastoma cells secrete VEGF and grow subcutaneously in athymic mice (Saleh, *et al.*, *Cancer Res.* **1996**, *56*, 393-401; Cheng, *et al.*, *Proc. Natl. Acad. Sci. USA* **1996**, *93*, 8502-8507). The introduction of antisense constructs to VEGF mRNA into these cell lines reduces their *in vivo* growth, as well as the degree of neovascularization. Monoclonal antibodies against VEGF inhibit the subcutaneous growth of human rhabdomyosarcoma, glioblastoma, leiomyosarcoma (Kim, *et al.*, *Nature* **1993**, *362*, 841-844) and fibrosarcoma (Asano, *et al.*, *Cancer Res.* **1995**, *55*, 5296-5301) in athymic mice. Metastasis of fibrosarcoma (Asano, *et al.*, *Cancer Res.* **1995**, *55*, 5296-5301) and colon cancer tumors (Warren, *et al.*, *J. Clin. Invest.* 1995, *95*, 1789-1797) was also blocked by anti-VEGF antibodies. The presence of VEGF in primary tumors and tumor cell lines, as well as the inhibitory activity of antisense and neutralizing antibodies to VEGF, indicate that VEGF is a significant player in tumor angiogenesis.

The contribution of the VEGF receptors, Flk-1/KDR and Flt-1, to tumor growth has also been studied extensively. Like VEGF, their mRNA has been detected in tumors such as gliomas (Plate, *et al.*, *Lab Invest.* **1992**, *67*, 529-534), Plate, *et al.*, *Int. J. Cancer* **1994**, *59*, 520-529), hemangioblastomas (Hatva, *et al.*, *Am. J. Pathol.* **1996**, *146*, 368-378), colon cancer (Takahashi, *et al.*, *Cancer Res.* **1995**, *55*, 3964-3968) and adenocarcinomas (Brown, *et al.*, *Cancer Res.* **1993**, *53*, 4727-4735). In these cases, the receptors were detected on the endothelial cells of the vessels and not the tumor cells. This supports a paracrine mechanism in which VEGF secreted from tumor cells stimulates

proliferation of endothelial cells. In contrast, Kaposi Sarcoma (KS) cell lines and primary tissue express Flt-1 and KDR as well as VEGF (Masood, *et al., Proc. Natl. Acad. Sci. USA* **1997**, *94*, 979-984). This finding, coupled with the observation that growth of KS cell lines is inhibited by VEGF antisense oligonucleotides, suggests that an autocrine loop may be present in Kaposi Sarcoma leading to enhanced cell growth and vasculature.

The capacity of Flk-1 to act as a modulator of tumor growth has also been studied in animal tumor models. Athymic mice were co-implanted with tumor cells and virus-producing cells that produced viral DNA encoding a truncated *flk-1* gene (Millauer, *et al.*, *Nature* **1994**, *367*, 576-579; Millauer, *Cancer Res.* **1996**, *56*, 1615-1620). The co-implantation allowed the introduction of mutant receptor into endothelial cells where it acted in a dominant-negative fashion to block activation of Flk-1 and effect the growth of the tumor. By this method, the subcutaneous growth of a variety of human, rat and mouse tumor cells was shown to be inhibited. In addition, the microvessel density was shown to be reduced in the small tumors that did form, confirming the connection between Flk-1, angiogenesis, and tumor growth.

Flk-1/KDR is an excellent target for the development of novel anticancer agents. Specific inhibitors of Flk-1/KDR would be expected to have fewer side effects than cytotoxic chemotherapy drugs, since angiogenesis is thought to rarely occur in healthy adults (with the exception of angiogenesis that occurs following wound injury or during cyclical changes in the endometrium and ovary). Monoclonal antibodies specific for VEGF (Kim, *et al.*, *Nature* **1993**, *362*, 841-844) and Flk-1 (Rockwell, *et al.*, *Proc. Am. Assc. Cancer Res.* **1997**, *38*, 266) have been shown to inhibit tumor growth in animals by disrupting binding of VEGF to the receptor. Also, synthetic small molecule inhibitors of Flk-1 tyrosine kinase activity have been shown to block the effects of VEGF in several *in vitro* and *in vivo* systems (Strawn, *et al.*, *Cancer Res.* **1996**, *56*, 3540-3545).

Acidic and basic fibroblast growth factors (FGF1/aFGF and FGF2/bFGF) and their receptors, FGFR-1 and FGFR-2, have been identified in a variety of tumor types. A human renal cell carcinoma cell line has been shown to secrete FGF2/bFGF (Singh, *et al.*, *Cell Growth Diff.* **1996**, *7*, 397-404) and two human prostate tumor cell lines were found to make and respond to FGF2/bFGF (Nakamoto, *et al.*, *Cancer Res.* **1992**, *52*, 571-577). Analysis of mRNA from various grades of astrocytomas revealed that the expression of different FGF receptors changes as the tumors progress to higher grades of malignancy

(Yamaguchi, *et al.*, *Proc. Natl. Acad. Sci. USA* **1994**, *91*, 484-488). Surgically excised skin from melanoma patients was found to have high FGFR-1 expression in the invading melanoma cells and stroma, but not in the endothelial cells (Kaipainen, *et al.*, *Cancer Res.* **1994**, *54*, 6571-6577). The distribution pattern of FGFs and their receptors implicate FGFs in tumor growth.

Neutralizing antibodies specific for FGF2/bFGF have been used to investigate the role of FGF2/bFGF in cancer. FGF-induced mitogenesis of SC115 mouse mammary carcinoma cells in response to bFGF was shown to be inhibited by anti-FGF antibodies (Lu, *et al.*, *Cancer Res.* **1989**, *49*, 4963-4967). Similarly, an anti-FGF2/bFGF monoclonal antibody blocked the growth of U-87MG and T98G human glioblastoma cells in culture and as xenografts in nude mice (Takahashi, *et al.*, *FEBS Lett.* **1991**, *288*, 65-71).

Other growth factors and their receptors that play a supporting role in angiogenesis are probably also involved in tumor growth. PDGF receptors have also been identified in various tumors and tumor cell lines and contribute to the transformation of cells. As with the FGF receptors, they may contribute to the growth of tumors by promoting angiogenesis and tumor cell proliferation and survival.

The PDGF and PDGF-receptor family should be considered an important player in angiogenic studies particularly in light of the recent knock-out mice showing a role for PDGF-B chain in pericytes. PDGF is the most potent mitogen for cells of mesenchymal origin. Kidney glomerular mesangial cells were the targets of disrupted PDGF-B or PDGF-ß receptor (Levéen, P., *et al.*, *Genes Dev.* **1994**, *8*, 1875-1887; Soriano, P., *Genes Dev.* **1994**, *8*, 1888-1896) genes in mice and were found to lead to the development of lethal hemorrhage and edema in late embryogenesis. Mesangial cells are related to microvascular pericytes, another target of the disrupted PDGF-B gene. Pericytes encircle the microvessels in many different tissues. They are contractile cells and therefore may contribute to the mechanical stability of the capillary wall. Pericytes express PDGF receptors and respond to PDGF *in vitro*. Pericytes may also regulate endothelial cell function. Thus, PDGF and its receptors may function to support vascular integrity.

Rheumatoid Arthritis

Rheumatoid arthritis (RA) is an inflammatory joint disease that is characterized by cellular infiltration of synovial fluid by neutrophils, and of the synovial membrane by T

lymphocytes and macrophages, hyperproliferation of cells of the synovial membrane which results in formation of a pannus, and destruction of cartilage and bone (Feldman, *et al.*, *Ann. Rev. Immunol.* **1996**, *14*, 397-440; Paleolog, *Br. J. Rheumatol.* **1996**, *35*, 917-920). Angiogenesis is thought to have an important role in the pathogenesis of RA (Colville-Nash & Scott, *Annals. Rheumatic Diseases* **1992**, *51*, 919-925, and references therein); inhibition of angiogenesis with the fumagillin analog, AGM-1470, suppresses RA in experimental models of RA (Peacock, *et al.*, *J. Exp. Med.* **1992**, *175*, 1135-1138; Peacock, *et al.*, *Cell. Immunol.* **1995**, *160*, 178-184).

Many growth factors and cytokines have been implicated as having a role in RA (*see* Feldman, *et al.*, *Ann. Rev. Immunol.* **1996**, *14*, 397-440 for a review), some of which have also been implicated as angiogenic factors (see Colville-Nash & Scott, *Ann. Rheumatic Dis.* **1992**, *51*, 919-925; Shawver, *et al.*, *Drug Disc. Today* **1997**, *2*, 50-63 for reviews). Putative angiogenic factors that have been reported to be expressed in RA synovial fluid or tissue include VEGF (Fava, *et al.*, *J. Exp. Med.* **1994**, *180*, 341-346; Koch, *et al. J. Immunol.* **1994**, *152*, 4149-4156; Nagashima, *et al.*, *J. Rheumatol.* **1995**, *22*, 1624-1630; Ben-Av, *et al.*, *FEBS Lett.* **1995**, *372*, 83-87), FGF2/FGF2/bFGF (Cozzolino, *et al.*, *J. Clin. Invest.* **1993**, *91*, 2504-2512; Tamura, *et al.*, *Endocrinol* **1996**, *137*, 3729-3737), and FGF1/aFGF (Byrd, *et al.*, *Arth. & Rheumat.* **1996**, *39*, 914-922). The strongest evidence for a role as a direct angiogenic factor exists for VEGF.

VEGF expression is significantly higher in synovial fluid and tissue from RA patients than from patients with other types of arthritis (Fava, *et al.*, *J. Exp. Med.* **1994**, *180*, 341-346; Koch, *et al. J. Immunol.* **1994**, *152*, 4149-4156). The source of this VEGF appears to be elevated expression in synovial lining cells, subsynovial macrophages, fibroblasts surrounding microvessels, and vascular smooth muscle cells (Fava, *et al.*, *J. Exp. Med.* **1994**, *180*, 341-346; Koch, *et al. J. Immunol.* **1994**, *152*, 4149-4156; Nagashima, *et al.*, *J. Rheumatol.* **1995**, *22*, 1624-1630). Indirect induction of VEGF by other factors may occur as well.

Synovial lining cells, macrophages, endothelial cells, and vascular smooth muscle cells of rheumatoid joints (Hosaka, *et al.*, *Pathobiol.* **1995**, *63*, 249-56), as well as mast cells in rheumatoid synovium (Qu, *et al.*, *Am. J. Pathol.* **1995**, *147*, 564-573), have been reported to express FGF2/bFGF, but it does not appear to be elevated in RA synovial fluid (Hosaka, **1995**, *supra*). FGF1/aFGF is abundantly expressed in synovial tissues from RA

patients (Byrd, *et al.*, *Arthritis & Rheumatism* **1996**, *39*, 914-922), and expression of FGF-R1, the receptor for FGF1 and FGF2 (Ornitz, *et al.*, *J. Biol. Chem.* **1996**, *271*, 15292-15297), is enhanced on $CD4^+$ T cells (Byrd, **1996**, *supra*).

Psoriasis

Psoriasis is a chronic skin disorder that is characterized by hyperproliferation of the epidermis, inflammation, and angiogenesis. Angiogenesis appears to be crucial in the pathogenesis of psoriasis, and microvascular changes are one of the earliest detectable events in developing psoriatic lesions (for a review *see* Creamer & Barker, *Clin. Exp. Dermatol.* **1995**, *20*, 6-9). Several reports have implicated the epidermis as the origin of angiogenic factors (Nishioka & Ryan, *J. Invest. Dermatol.* **1972**, *58*, 33-45; Wolf & Harrison, *J. Invest. Dermatol.* **1973**, *59*, 40-43; Barnhill, *et al.*, *Br. J. Dermatol* **1984**, *110*, 273-281; Malhotra, *et al.*, *Lab. Invest.* **1989**, *61*, 162-165). However, it has long been recognized that the inflammatory component of the disease complicates dissection of the angiogenic factors involved in the disease (Wolf, *Lab. Invest.* **1989**, *61*, 139-142), since cytokines secreted by lymphocytes, macrophages, mast cells and neutrophils can also contribute to angiogenesis (Majewski, *et al.*, *Arch. Dermatol.* **1985**, *121*, 1018-1021; Majewski, *et al.*, *Arch. Dermatol.* **1987**, *123*, 221-225; Koch, *et al.*, *Science* **1992**, *258*, 1798-1801; Qu, *et al.*, *Am. J. Pathol.* **1995**, *147*, 564-573).

Of the many angiogenic factors identified in skin (Arbiser, Am. Acad. Derm. **1996**, *34*, 486-497), VEGF has been the best characterized as a direct inducer of angiogenesis. VEGF is overexpressed in keratinocytes of psoriatic skin, but only minimally expressed in normal epidermis (Detmar, *et al.*, *J. Exp. Med.* **1994**, *180*, 1141-1146). VEGF is also overexpressed in other skin diseases such as bullous pemphigoid, dermatitis herpetiformis, and erythema multiforme (Brown, *et al.*, *Invest. Dermatol.* **1995**, *104*, 744-749), in delayed skin hypersensitivity reactions (Brown, *et al.*, *J. Immunol.* **1995**, *154*, 2801-2807), and probably after sun exposure, as suggested by the induction of VEGF expression in cultured keratinocytes following exposure to ultraviolet light (Brauchle, *et al.*, *J. Biol. Chem.* **1996**, *271*, 21793-21797).

FGF2/bFGF is expressed in keratinocytes and endothelial cells and stimulates the proliferation of both through autocrine and paracrine mechanisms (Schweigerer, *et al.*, *Nature* **1987**, *325*, 257-259; O'Keefe, *et al.*, *J. Invest. Dermatol.* **1988**, *90*, 767-769). Mast

cells have also been reported to be a major source of FGF2/bFGF in chronic inflammatory diseases (Qu, *et al.*, *Am. J. Pathol.* **1995**, *147*, 564-573), which may contribute to the link between inflammation and angiogenesis.

Ocular Diseases

The release of angiogenic factors from the ischemic retina has been hypothesized to be the central stimulus for retinal neovascularization. Glaucoma, vitreous hemorrhage and retinal detachment, secondary to intraocular neovascularization, accounts for the resultant vision loss in several ocular disorders such as retinopathy of prematurity, age-related macular degeneration, and diabetic retinopathy. The release of angiogenic factors by the ischemic retina to induce new blood vessel growth and increase the oxygen supply to the area turns out to be harmful as the new vessels do not grow with normal architecture. Edema, hemorrhage, vessel tortuosity, and pathological neovascularization subsequently result in retinal detachment and lead to blindness.

Some angiogenic factors such as IGF-1, FGF and VEGF have elevated expression in vitreous and neovascular membranes from patients with retinal disorders (Grant, *et al.*, *Diabetes* **1986**, *35*, 416-20; Sivalingam, *et al.*, *Arch Ophthalmol.* **1990**, *108*, 869-72; Aiello, *et al.*, *New Engl. J. Med.* **1994**, *331*, 1480-1487; Malecaze, *et al.*, *Arch. Ophthalmol.* **1994**, *112*, 1476-82; Adamis, *et al.*, *Amer. J. Ophthalmology* **1994**, *118*, 445-50). Therefore, these growth factors are candidates for the angiogenic factors which modulate or initiate intraocular neovascularization in retinal disorders.

VEGF is constitutively expressed in the vascularized tissues of the normal eye (Adamis, *et al.*, *Arch. Ophthalmol.* **1996**, *114*, 66-71). Intraocular VEGF gene expression is increased in disease states like diabetic retinopathy (Adamis, *et al.*, *Amer. J. Ophthalmology* **1994**, *118*, 445-450; Malecaze, *et al.*, *Arch. Ophthalmology* **1994**, *112*, 1476-1482; Aiello, **1994**, *supra*; Pe'er, *et al.*, *Lab Invest.* **1995**, *72*, 638-645). Using a neutralizing antibody specific for VEGF and soluble VEGF-receptor chimeric proteins, it has been shown that VEGF is sufficient to induce neovascularization (Aiello, **1994**, *supra*; Adamis, **1994**, *supra*; Tolentino, *et al.*, *Ophthalmology* **1996**, *103*, 1820-1828; Tolentino, *et al.*, *Arch. Ophthalmol.* **1996**, *114*, 964-970). In animal models, intraocular injections of VEGF into normal eyes caused retinal edema, microaneurysms, hemorrhage and intraretinal neovascularization (Pierce, *et al.*, *Arch. Ophthalmol.* **1995**, *114*, 964-970;

Miller, *et al.*, *Am. J. Pathol.* **1994**, *145*, 574-584; Tolentino, **1996**, *supra*). Decreased levels of VEGF paralleled the regression of proliferative retinopathy. Stimuli associated with oxygen deprivation such as hypoxia (Pierce, **1995**, *supra*; Miller, **1994**, *supra*; Shweiki, *et al.*, *Nature* **1992**, *359*, 843-845; Plate, *et al.*, *Lab Invest.* **1992**, *67*, 529-34), generation of oxygen intermediates (Kuroki, *et al.*, *Angiogenesis Novel Therapeutic Development Conference* **1996**, unpublished communication), and accumulation of advanced glycation endproducts (Adamis, Angiogenesis Novel Therapeutic Development Conference, **1997**) in diabetics, increased endogenous VEGF expression *in vivo*. Inhibitors of VEGF signaling including antisense VEGF reagents (Smith, *IBC Conference on Angiogenesis Inhibitors and Other Novel Therapeutic Strategies for Ocular Diseases of Neovascularization* **1996**) and soluble VEGF receptor chimeric proteins (Aiello, *et al.*, *Proc. Natl. Acad. Sci. USA* **1995**, *92*, 10457-10461), ribozymes targeting VEGF receptor subtype mRNAs (Cushman, *et al.*, *Abstract from Angiogenesis Inhibitors and Other Novel Therapeutic Strategies for Ocular Diseases of Neovascularization* **1996**; Pavco, *IBC Conference on Novel Anti-angiogenic Therapy for Diabetic Retinopathy, Macular Degeneration and Other Ocular Diseases of Neovascularization* **1997**) and selective PKC inhibitors (Aiello, **1995**, *supra*) resulted in significant inhibition of corneal and retinal neovascularization, VEGF-stimulated cell growth *in vitro* and VEGF-induced retinal permeability *in vivo*. All of these data provide strong support for a direct role of VEGF in ocular angiogenesis.

FGF2/bFGF appears to play a role in diabetic retinopathy (Sivalingam, *et al.*, *Arch Ophthalmol* **1990**, *108*, 869-872; Hanneken, *et al.*, *Arch. Ophthalmol* **1991**, *109*, 1005-1011). In addition to being present in vitrectomy samples from retinal disorders (Sivalingam, **1990**, *supra*), FGF2/bFGF induces endothelial cell proliferation (Gospodarowitz, *Prog. Clin. Biol. Res.* **1976**, *9*, 1-19; D'Armore & Klagsbrun, *J. Cell Biol.* **1976**, *99*, 1545-9), migration (Herman & D'Armore, *J. Muscle Res. Cell Motil* **1984**, *5*, 697-709) and the release of collagenase and plasminogen activators (Presta, *et al.*, *Mol. Cell. Biol.* **1986**, *6*, 4060-6). *In vivo*, FGF2/bFGF induces corneal neovascularization (Gospodarowitz, *et al.*, *Exp. Eye Res.* **1979**, *28*, 501-14; Risau, *Proc. Natl. Acad Sci. USA* **1986**, *83*, 3855-3859) as well as retinal fibrovascular proliferation with an enhanced fibrotic component compared to IGF-1 when injected into the vitreous cavity (Grant, *et al.*, *Reg. Peptides* **1993**, *48*, 267-278). The development of retinal capillary basement

membrane thickening and subsequent retinal traction and detachment is similar to those occurring in humans and animals with diabetes.

Arterial Thickening and Restenosis

Arterial injury as part of the atherosclerotic process or as a consequence of balloon-mediated injury to treat coronary occlusions is known to be accompanied by thickening of the arterial wall. This response is also observed in cases of organ rejection where chronic vascular injury is associated with thickening of the arteries at the site of the organ transplant. RTKs have been implicated as important players within the disease process associated with the injury response. As in the case of atherosclerosis (herein), both PDGF and FGFs are associated with the hyperproliferation of the arterial smooth muscle cell. When these cell layers become ischemic, they require new blood vessel formation in order to support their hyperproliferation. In the case of smooth muscle cell migration, the activation of mitogen-activated protein kinase has been shown to be associated with PDGF-dependent cell movement (Graf, *et al., Hypertension* **1997**, *29*, 334-339).

A synthetic compound that blocks binding of PDGF to its receptor has been shown to inhibit chemotaxis of smooth muscle cells and inhibit neo-intimal formation in restenotic lesions following balloon-injury of carotid arteries in rats (Mullins, *et al., Arterioscler Thromb* **1994**, *14*, 1047-1055). In addition, PDGF mRNA levels have been shown to be increased in human cardiac allografts (Zhao, *et al., J. Clin. Invest.* **1994**, *94*, 992-1003). In animal models, PDGF and FGF have been shown to be expressed in the injury response following cardiac (Zhao, **1994**, *supra*; Zhao, *et al., Circulation* **1994**, *90*, 677-685) and renal (Alpers, *et al., Am. J. Pathol.* **1996**, *148*, 439-451; Abboud, *Annu. Rev. Physiol.* **1995**, *57*, 297-309) transplantation.

Atherosclerosis

Atherosclerosis is a disease associated with the formation of arterial lesions or atheromas consisting of endothelium-covered fibro-fatty plaques. Beneath the endothelial layer exists smooth muscle cells and extracellular matrix components containing variable amounts of serum proteins. This overlies an area characterized by collections of lipid-laden macrophages. Significant numbers of lymphocytes, particularly T cells, are also

present and may contribute to lymphocyte-mediated angiogenesis (Kaminski, M. & Auerbach, R., *Proc. Soc. Exp. Biol. Med.* **1988**, *188*. 440-443). Both macrophages and lymphocytes traverse the endothelium in order to enter the atheroma lesion. The lesion is characterized by a flux of blood cells including platelets that enter or exit the endothelium. Similar to the restenotic lesion, the build up of smooth muscle cells is supported by angiogenesis and is likely to be stimulated by factors released from the smooth muscle cells when they become hypoxic. In contrast to atheromas, thrombi of arteries and veins are characterized by structures containing a fibrin mesh in which blood cells are entrapped.

The presence of platelets and other cells in the lesion have led to a number of studies that implicate growth factors and their cognate receptor tyrosine kinases as players in the disease (Chabrier, *Int. Angiol.* **1996**, *15*, 100-103). The most prominent player in this regard is PDGF. PDGF has been detected in atherosclerotic lesions of rat (Waltenbeger, *et al., Arterioscler Thromb. Vasc. Biol* **1996**, *16*, 1516-1523), rabbit (Agapitos, *et al., Int. Angiol.* **1996**, *15*, 249-251), and human (Billett, *et al., Arterioscler Thromb. Vasc. Biol.* **1996**, *16*, 399-406; Ito, *et al., Neurol. Res.* **1995**, *17*, 345-348) origin. The release of PDGF from platelets and other lymphocytes is thought to elicit a pleiotrophy of paracrine effects on blood cells and smooth muscle cells in the vicinity of the lesion. The chemoattractant, cell survival, migratory and mitogenic properties of PDGF receptor function in cells may contribute directly or indirectly to different activities on different cell types.

PDGF RNA expression has been shown to be associated with the presence of circulating mononuclear cells in hypercholesterolemic patients (Billett, **1996**, *supra*). The presence of mononuclear and other blood cells in the atherosclerotic lesion has been shown to also elaborate the expression of VEGF and FGF growth factors. In smooth muscle cells, PDGF has been shown to exert a number of effects that may directly or indirectly effect the angiogenic process (Newby, A.C. & George, S.J., *Curr. Opin. Cardiol.* **1996**, *11*, 574-582). For instance, it has been suggested that PDGF signaling may upregulate the expression of VEGF in smooth muscle cells (Stavri, *et al., FEBS Lett.* **1995**, *358*, 311-315). In addition, it is clear that PDGF is a player in myointimal proliferation associated with formation of atherosclerotic plaques. The proliferative (Randone, *et al., Eur. J. Vasc. Endovasc. Surg.* **1997**, *13*, 66-71), migratory (Graf, **1997**, *supra*; Abedi, H.

& Zachary, I., *Cardiovasc. Res.* **1995**, *30*, 544-56; Koster, *et al.*, *Angiology* **1995**, *46*, 99-106), and cell survival (Bennet, *et al.*, *J. Clin. Invest.* **1995**, *95*, 2266-2274) aspects of PDGF function in smooth muscle cells may be a major determinant of the formation of the atherosclerotic lesion.

In addition, increased expression of PDGF (Waltenberger, **1996**, *supra*; Stavri, *1995, supra*; Michiels, *et al., Exp. Cell. Res.* **1994**, *213*, 43-54), VEGF (Stavri, *1995, supra*; Knighton, *et al., Science* **1983**, *221*, 1283-1285; Li, *et al., Am. J. Physiol.* **1996**, *270*, 1803-1811), and FGF (Michiels, **1994**, *supra*) has been shown to occur in smooth muscle cells under conditions of hypoxia. It has been well established that endothelial cell growth and angiogenesis is triggered by conditions of low oxygen tension. The inducibility of these factors is consistent with previous association of these factors and Flk-1/KDR, Flt-1, PDGF receptors, and FGF receptors as participants in this process. In the case of a fibrotic thrombus, the fibrin gel itself is viewed as a provisional matrix into which vascularized connective tissue invades, similar to wound healing. FGF has been considered to be an important player in hyperproliferative and angiogenic aspects of this lesion. FGF1/aFGF and FGF2/bFGF or receptor expression has been shown to be increased in rat aortic smooth muscle cells (van Neck, *et al., Biochim. Biophys. Acta.* **1995**, *1261*, 210-214), human cardiac allografts (Zhao, **1994**, *supra*; Zhao, **1994**, *supra*), CD4+ T cells (Zhao, *et al., J Immunol.* **1995**, *155*, 3904-3911), and atherosclerotic human arteries (Hughes, *Cardiovasc. Res.* **1996**, *32*, 557-569).

Since PDGF, VEGF and FGF play a role in the formation of atheromas and fibrotic lesions, RTK-mediated signaling events may be important in the development and maintenance of the lesion. Interruption of these signaling systems inhibit the formation or disrupt the remodeling or integrity of the lesion. In the fibrotic thrombus, targeting of these receptors may be advantageous to reduce the number of connective tissues cells involved in fibrin deposition and development of the clot.

Tissue Ischemia

The formation of new blood vessels is tightly regulated by receptor tyrosine kinases and their cognate ligands. This has been best substantiated for the VEGF, FGF and PDGF receptor systems where these receptors effect the growth and survival of endothelial cells, pericytes, and arterial smooth muscle cells. In the case of tissue

ischemia, the induction of new blood vessel growth may be advantageous for the treatment of specific human diseases where oxygen and nutrient limitation is linked to the disease pathology. Since PDGF (Alpers, **1996**, *supra*; Waltenbeger, **1996**, *supra*; Michiels, **1994**, *supra*), VEGF (Stavri, **1995**, *supra*; Knighton, **1983**, *supra*; Li, **1996**, *supra*), and FGF (Michiels, **1994**, *supra*) have been shown to be expressed in cells under hypoxic conditions, growth factor therapy leading to the induction of new blood vessels has been suggested as a mechanism to overcome myocardial ischemia (Ware & Simons, *Nature Med* **1997**, *3*, 158-164). This rationale suggests that treatment for diseased hearts may relieve symptoms by restoration of myocardial oxygen or restoration of blood flow and thereby prevent disease progression or improve treatments using angioplasty or following coronary bypass surgery.

Animal experiments support the rationale for treatments based on these factors. Acute ischemia models in dogs (Yanigisawa-Miwa, *et al., Science* **1992**, *257*, 1401-1403) and pigs (Battler, *et al., J. Am. Coll. Cardio.* **1995**, *22*, 2001-2006; Padua, *et al., Mol. Cell. Biochem.* **1995**, *143*, 129-135) have suggested that intracoronary injection of FGF may be beneficial. Chronic administration of FGF2/bFGF to dogs with chronic coronary occlusion showed a fast improvement in function and increased number of vessels (Giordano, *et al., Nature Med.* **1996**, *2*, 534-539; Lazarous, *et al., Circulation* **1995**, *91*, 145-153). Moreover, chronic intracoronary infusion of VEGF resulted in substantial improvement of coronary flow of the diseased heart (Pearlman, *et al., Nature Med.* **1995**, *1*, 1085-1089; Harada, *et al., Am. J. Physiol.* **1996**, *270*, 1792-1802). Peripheral ischemia has been shown to have improved collateral circulation upon injection of FGF2/bFGF (Baffour, *et al., J. Vasc. Surg.* **1992**, *16*, 181-191) and VEGF (Takeshita, *et al., J. Clin. Invest* **1994**, *93*, 662-670). More recently, VEGF gene therapy trials have been initiated to measure improved vascular function (Isner, *Lancet* **1996**, *348*, 370-374).

Endometriosis

Endometriosis also requires angiogenesis and neovascularization to permit the establishment and growth of endometriotic cells outside the uterus. Endometriosis is the most prevalent cause of female infertility, and affects approximately 6 million women in the U.S. The disease is most commonly characterized by the growth of endometrium in the peritoneal cavity where it proliferates, invades, secretes, desquamates, and

accumulates with each ovarian cycle. Abdominal pain, fibrosis, rare ascites formation, and adhesions of intra-abdominal structures, result.

Endometriosis has been linked to increased levels of angiogenic factors and specifically to elevated VEGF levels (L. Brown *et al.*, *Lab. Invest.*, **1997**, *76*, 245-255; J. Shifren *et al.*, *J. Clin. Endocrinol. Metab.*, **1996**, *81*, 3112-3118; J. McLaren *et al.*, *Hum. Reprod.*, **1996**, *11*, 220-223). During the menstrual cycle, expression of Flt is constant but that of kinase insert domain containing receptor (FLK-1/KDR) is increased in the luteal phase, at which time the cells migrate in response to VEGF. FLK-1/KDR expression and the migratory response are significantly higher in patients with endometriosis (J. McLaren, *et al.*, *J. Clin. Invest.* **1996**, *98*, 482). Thus it is likely that the methods of the invention will be able to prevent or inhibit the growth of endometriotic cells outside the uterus.

Thus, as the above passages show, the compounds of the invention can be used for the treatment of several kinds of diseases which include, but not limited to, cancers, such as, squamous cell carcinoma, astrocytoma, glioblastoma, lung cancer, bladder cancer, head and neck cancer, melanoma, ovarian cancer, prostate cancer, breast cancer, gastrointestinal cancer, colorectal cancer, small-cell lung cancer and glioma, and immunological disorders, hyperproliferation disorders, cardiovascular disorders, inflammatory disorders, restenosis, fibrosis, psoriasis, osteoarthritis, rheumatoid arthritis, atherosclerosis, diabetes, and angiogenesis.

II. Pharmaceutical Formulations And Routes Of Administration

The compounds described herein can be administered to a human patient *per se*, or in pharmaceutical compositions where it is mixed with other active ingredients, as in combination therapy, or suitable carriers or excipient(s). Techniques for formulation and administration of the compounds of the instant application may be found in "Remington's Pharmaceutical Sciences," Mack Publishing Co., Easton, PA, latest edition.

a) Routes Of Administration.

Suitable routes of administration may, for example, include oral, rectal, transmucosal, or intestinal administration; parenteral delivery, including intramuscular, subcutaneous, intravenous, intramedullary injections, as well as intrathecal, direct intraventricular, intraperitoneal, intranasal, or intraocular injections.

Alternately, one may administer the compound in a local rather than systemic manner, for example, via injection of the compound directly into a solid tumor, often in a depot or sustained release formulation.

Furthermore, one may administer the drug in a targeted drug delivery system, for example, in a liposome coated with tumor-specific antibody. The liposomes will be targeted to and taken up selectively by the tumor.

b) Composition/Formulation.

The pharmaceutical compositions of the present invention may be manufactured in a manner that is itself known, *e.g.*, by means of conventional mixing, dissolving, granulating, dragee-making, levigating, emulsifying, encapsulating, entrapping or lyophilizing processes.

Pharmaceutical compositions for use in accordance with the present invention thus may be formulated in conventional manner using one or more physiologically acceptable carriers comprising excipients and auxiliaries which facilitate processing of the active compounds into preparations which can be used pharmaceutically. Proper formulation is dependent upon the route of administration chosen.

For injection, the agents of the invention may be formulated in aqueous solutions, preferably in physiologically compatible buffers such as Hanks's solution, Ringer's solution, or physiological saline buffer. For transmucosal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are generally known in the art.

For oral administration, the compounds can be formulated readily by combining the active compounds with pharmaceutically acceptable carriers well known in the art. Such carriers enable the compounds of the invention to be formulated as tablets, pills, dragees, capsules, liquids, gels, syrups, slurries, suspensions and the like, for oral ingestion by a patient to be treated. Pharmaceutical preparations for oral use can be obtained solid excipient, optionally grinding a resulting mixture, and processing the mixture of granules, after adding suitable auxiliaries, if desired, to obtain tablets or dragee cores. Suitable excipients are, in particular, fillers such as sugars, including lactose, sucrose, mannitol, or sorbitol; cellulose preparations such as, for example, maize starch, wheat starch, rice starch, potato starch, gelatin, gum tragacanth, methyl cellulose,

hydroxypropylmethyl-cellulose, sodium carboxymethylcellulose, and/or polyvinylpyrrolidone (PVP). If desired, disintegrating agents may be added, such as the cross-linked polyvinyl pyrrolidone, agar, or alginic acid or a salt thereof such as sodium alginate.

Dragee cores are provided with suitable coatings. For this purpose, concentrated sugar solutions may be used, which may optionally contain gum arabic, talc, polyvinyl pyrrolidone, carbopol gel, polyethylene glycol, and/or titanium dioxide, lacquer solutions, and suitable organic solvents or solvent mixtures. Dyestuffs or pigments may be added to the tablets or dragee coatings for identification or to characterize different combinations of active compound doses.

Pharmaceutical preparations which can be used orally include push-fit capsules made of gelatin, as well as soft, sealed capsules made of gelatin and a plasticizer, such as glycerol or sorbitol. The push-fit capsules can contain the active ingredients in admixture with filler such as lactose, binders such as starches, and/or lubricants such as talc or magnesium stearate and, optionally, stabilizers. In soft capsules, the active compounds may be dissolved or suspended in suitable liquids, such as fatty oils, liquid paraffin, or liquid polyethylene glycols. In addition, stabilizers may be added. All formulations for oral administration should be in dosages suitable for such administration.

For buccal administration, the compositions may take the form of tablets or lozenges formulated in conventional manner.

For administration by inhalation, the compounds for use according to the present invention are conveniently delivered in the form of an aerosol spray presentation from pressurized packs or a nebuliser, with the use of a suitable propellant, *e.g.*, dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane, carbon dioxide or other suitable gas. In the case of a pressurized aerosol the dosage unit may be determined by providing a valve to deliver a metered amount. Capsules and cartridges of *e.g.* gelatin for use in an inhaler or insufflator may be formulated containing a powder mix of the compound and a suitable powder base such as lactose or starch.

The compounds may be formulated for parenteral administration by injection, *e.g.*, by bolus injection or continuous infusion. Formulations for injection may be presented in unit dosage form, *e.g.*, in ampoules or in multi-dose containers, with an added preservative. The compositions may take such forms as suspensions, solutions or

emulsions in oily or aqueous vehicles, and may contain formulatory agents such as suspending, stabilizing and/or dispersing agents.

Pharmaceutical formulations for parenteral administration include aqueous solutions of the active compounds in water-soluble form. Additionally, suspensions of the active compounds may be prepared as appropriate oily injection suspensions. Suitable lipophilic solvents or vehicles include fatty oils such as sesame oil, or synthetic fatty acid esters, such as ethyl oleate or triglycerides, or liposomes. Aqueous injection suspensions may contain substances which increase the viscosity of the suspension, such as sodium carboxymethyl cellulose, sorbitol, or dextran. Optionally, the suspension may also contain suitable stabilizers or agents which increase the solubility of the compounds to allow for the preparation of highly concentrated solutions.

Alternatively, the active ingredient may be in powder form for constitution with a suitable vehicle, *e.g.*, sterile pyrogen-free water, before use.

The compounds may also be formulated in rectal compositions such as suppositories or retention enemas, *e.g.*, containing conventional suppository bases such as cocoa butter or other glycerides.

In addition to the formulations described previously, the compounds may also be formulated as a depot preparation. Such long acting formulations may be administered by implantation (for example subcutaneously or intramuscularly) or by intramuscular injection. Thus, for example, the compounds may be formulated with suitable polymeric or hydrophobic materials (for example as an emulsion in an acceptable oil) or ion exchange resins, or as sparingly soluble derivatives, for example, as a sparingly soluble salt.

A pharmaceutical carrier for the hydrophobic compounds of the invention is a cosolvent system comprising benzyl alcohol, a nonpolar surfactant, a water-miscible organic polymer, and an aqueous phase. The cosolvent system may be the VPD co-solvent system. VPD is a solution of 3% w/v benzyl alcohol, 8% w/v of the nonpolar surfactant polysorbate 80, and 65% w/v polyethylene glycol 300, made up to volume in absolute ethanol. The VPD co-solvent system (VPD:D5W) consists of VPD diluted 1:1 with a 5% dextrose in water solution. This co-solvent system dissolves hydrophobic compounds well, and itself produces low toxicity upon systemic administration. Naturally, the proportions of a co-solvent system may be varied considerably without

destroying its solubility and toxicity characteristics. Furthermore, the identity of the co-solvent components may be varied: for example, other low-toxicity nonpolar surfactants may be used instead of polysorbate 80; the fraction size of polyethylene glycol may be varied; other biocompatible polymers may replace polyethylene glycol, *e.g.* polyvinyl pyrrolidone; and other sugars or polysaccharides may substitute for dextrose.

Alternatively, other delivery systems for hydrophobic pharmaceutical compounds may be employed. Liposomes and emulsions are well known examples of delivery vehicles or carriers for hydrophobic drugs. Certain organic solvents such as dimethylsulfoxide also may be employed, although usually at the cost of greater toxicity. Additionally, the compounds may be delivered using a sustained-release system, such as semipermeable matrices of solid hydrophobic polymers containing the therapeutic agent. Various of sustained-release materials have been established and are well known by those skilled in the art. Sustained-release capsules may, depending on their chemical nature, release the compounds for a few weeks up to over 100 days. Depending on the chemical nature and the biological stability of the therapeutic reagent, additional strategies for protein stabilization may be employed.

The pharmaceutical compositions also may comprise suitable solid or gel phase carriers or excipients. Examples of such carriers or excipients include but are not limited to calcium carbonate, calcium phosphate, various sugars, starches, cellulose derivatives, gelatin, and polymers such as polyethylene glycols.

Many of the PK modulating compounds of the invention may be provided as salts with pharmaceutically compatible counterions. Pharmaceutically compatible salts may be formed with many acids, including but not limited to hydrochloric, sulfuric, acetic, lactic, tartaric, malic, succinic, etc. Salts tend to be more soluble in aqueous or other protonic solvents that are the corresponding free base forms.

c) Effective Dosage.

Pharmaceutical compositions suitable for use in the present invention include compositions where the active ingredients are contained in an amount effective to achieve its intended purpose. More specifically, a therapeutically effective amount means an amount of compound effective to prevent, alleviate or ameliorate symptoms of disease or prolong the survival of the subject being treated. Determination of a therapeutically

effective amount is well within the capability of those skilled in the art, especially in light of the detailed disclosure provided herein.

For any compound used in the methods of the invention, the therapeutically effective dose can be estimated initially from cell culture assays. For example, a dose can be formulated in animal models to achieve a circulating concentration range that includes the IC_{50} as determined in cell culture (*i.e.*, the concentration of the test compound which achieves a half-maximal inhibition of the PK activity). Such information can be used to more accurately determine useful doses in humans.

Toxicity and therapeutic efficacy of the compounds described herein can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, *e.g.*, for determining the LD_{50} (the dose lethal to 50% of the population) and the ED_{50} (the dose therapeutically effective in 50% of the population). The dose ratio between toxic and therapeutic effects is the therapeutic index and it can be expressed as the ratio between LD_{50} and ED_{50}. Compounds which exhibit high therapeutic indices are preferred. The data obtained from these cell culture assays and animal studies can be used in formulating a range of dosage for use in human. The dosage of such compounds lies preferably within a range of circulating concentrations that include the ED_{50} with little or no toxicity. The dosage may vary within this range depending upon the dosage form employed and the route of administration utilized. The exact formulation, route of administration and dosage can be chosen by the individual physician in view of the patient's condition. (See *e.g.*, Fingl *et al.*, 1975, in "The Pharmacological Basis of Therapeutics", Ch. 1 p.1).

Dosage amount and interval may be adjusted individually to provide plasma levels of the active moiety which are sufficient to maintain the kinase modulating effects, or minimal effective concentration (MEC). The MEC will vary for each compound but can be estimated from *in vitro* data; *e.g.*, the concentration necessary to achieve 50-90% inhibition of the kinase using the assays described herein. Dosages necessary to achieve the MEC will depend on individual characteristics and route of administration. However, HPLC assays or bioassays can be used to determine plasma concentrations.

Dosage intervals can also be determined using MEC value. Compounds should be administered using a regimen which maintains plasma levels above the MEC for 10-90% of the time, preferably between 30-90% and most preferably between 50-90%.

In cases of local administration or selective uptake, the effective local concentration of the drug may not be related to plasma concentration.

The amount of composition administered will, of course, be dependent on the subject being treated, on the subject's weight, the severity of the affliction, the manner of administration and the judgment of the prescribing physician.

d) Packaging

The compositions may, if desired, be presented in a pack or dispenser device which may contain one or more unit dosage forms containing the active ingredient. The pack may for example comprise metal or plastic foil, such as a blister pack. The pack or dispenser device may be accompanied by instructions for administration. The pack or dispenser may also be accompanied with a notice associated with the container in form prescribed by a governmental agency regulating the manufacture, use, or sale of pharmaceuticals, which notice is reflective of approval by the agency of the form of the polynucleotide for human or veterinary administration. Such notice, for example, may be the labeling approved by the U.S. Food and Drug Administration for prescription drugs, or the approved product insert. Compositions comprising a compound of the invention formulated in a compatible pharmaceutical carrier may also be prepared, placed in an appropriate container, and labeled for treatment of an indicated condition. Suitable conditions indicated on the label may include treatment of a tumor, inhibition of angiogenesis, treatment of fibrosis, diabetes, and the like.

III. Enhancement of Sexual Activity

The present invention also relates to a method of increasing sexual function in mammals by administering to the mammal a compound of the invention. In some embodiments, the present invention involves monitoring the effect of the compound on the mammal. Methods of synthesis of the compounds of the invention, methods of administration of those compounds to mammals, methods of preparing pharmaceutical compositions containing those compounds, and some biological activities of those compounds are described in detail in the International Application No. PCT/US98/04134, by Shenoy et al., entitled "FORMULATIONS FOR HYDROPHOBIC PHARMACEUTICAL AGENTS",(Lyon & Lyon Docket No. 231/299), and U.S.

Provisional Application Serial No. 60/041,251, by Shenoy et al., entitled "FORMULATIONS FOR INDOLINONE COMPOUNDS", and filed March 18, 1997 (Lyon & Lyon Docket No. 224/266), both of which are incorporated herein by reference in their entirety, including any drawings.

EXAMPLES

The examples below are non-limiting and are merely representative of various aspects and features of the present invention. The examples describe methods for synthesizing compounds of the invention and methods for measuring an effect of a compound on the function of protein kinases.

The cells used in the methods are commercially available. The nucleic acid vectors harbored by the cells are also commercially available and the sequences of genes for the various protein kinases are readily accessible in sequence data banks. Thus, a person of ordinary skill in the art can readily recreate the cell lines in a timely manner by combining the commercially available cells, the commercially available nucleic acid vectors, and the protein kinase genes using techniques readily available to persons of ordinary skill in the art.

SYTHETIC PROCEDURES

EXAMPLE 1: PROCEDURES FOR SYNTHESIZING THE TRICYCLIC-BASED INDOLINONE COMPOUNDS OF THE INVENTION

General Synthesis Protocols for the tricyclic-based indolinone compounds of the invention:

The compounds are prepared by condensation of any one of the aldehyde/ketone/lactone compounds of Table 8 and any one of the tricyclic based indolin-2-ones, depicted in formulae VII, VIII, and IX, as described herein.

If aldehyde is one of the starting materials for preparing the compounds, Method 1 should be used. If ketone or lactone is one of the starting materials, Method 2 should be used for preparing the final product.

Method 1:

A reaction mixture of the proper tricyclic indolin-2-ones (1.0 equiv.), the appropriate aldehyde (1.2 equiv.), and piperidine (0.1 equiv.) in ethanol (1-2 mL/1.0 mmol oxindole) is stirred at 90 °C for 3-5 h. After cooling, the precipitate is filtered, washed with cold ethanol, and dried to yield the target compound.

Method 2:

The reaction mixture of the proper tricyclic indolin-2-ones (3.0 equiv.), the appropriate ketones or lactones (1.0 equiv.), and piperidine (3.0 equiv.) in DMF (1-2 mL/1.0 mmol ketones or lactones) is stirred at 130 °C for 5h. After cooling, the reaction mixture is poured into ice water. The following work-up procedures could be used depending on the nature of the products:

(a) The precipitate is filtered, washed with water, triturated with the mixture of ethyl acetate and hexane, and dried in oven for overnight.

(b) If the precipitate is too fine to be filtered, it is extracted with ethyl acetate. The organic layer is washed with brine, dried over sodium sulfate, and concentrated. The residue is then triturated with the mixture of ethyl acetate and hexane and dried in oven for overnight.

Preparation of Tricyclic based indolin-2-ones:

Oxindole of formula VII:

A thorough procedure may be found in *J. Chem. Soc.* **1913**, *103*, 1973-1985.

Oxindole of formula VIII and Oxindole of formula IX:

These two compounds could be easily prepared by the known literature procedure (*Synthesis,* **1993**, 51-53) from the commercially available starting materials (1,2-dichloro-3-nitro-naphthalene for the preparation of the oxindole of formula VIII and 6-chloro-7-nitro-quinoxaline for the synthesis of the oxindole of formula IX).

Preparation of the Aldehydes:

Most of the aldehydes, all of the ketones and lactones are commercially available. Aldehydes which are not commercially available could be prepared by conventional Vilsmeier formylation (*See*, for example, Org. Synth. Coll., Vol. IV, 1963, 831).

EXAMPLE 2: PROCEDURES FOR SYNTHESIZING THE PYRAZOLYLAMIDE-BASED COMPOUNDS OF THE INVENTION

The following procedures are given for the synthesis of the pyrazolylamide-based compounds of the invention. The names of the compounds of the invention hereby designated as AP-001 through AP-033 are given below.

Experimental:

For compounds AP-001 to AP-029:

General procedure:

To 1.2 eqivalents of 1-benzyl-3-tert-butylpyrazole-5-carbonyl chloride (Ref.: J. Gen. Chem. USSR, English Translation, Vol. 30, 1960, p 2901 or commercially available from Maybridge Chemical Company, UK) in 0.2 M solution of dichloromethane at room temperature is added 1 equivalent of the amine followed by 2 equivalents of diisopropylethylamine. The reaction is stirred at room temperature or heated at 55 °C if needed until the completion of the reaction. The reaction mixture is then diluted with water and extracted with ethyl acetate. The organic layer is then washed with saturated sodium bicarbonate solution, brine, dried over sodium sulfate, filtered, and concentrated. The crude reaction mixture is then purified on a silica gel column, using a solvent mixture of hexane/ethyl acetate or dichloromethane/methanol as the eluent.

Compound AP-001:

To a solution of 331 mg of 1-benzyl-3-tert-butylpyrazole-5-carbonyl chloride in 5 mL of dichloromethane at room temperature was added 161 mg of 4-trifluoromethylaniline followed by 0.6 mL of diisopropylethylamine. The mixture was then heated at 55 °C for 24 hrs. After dilution with 50 mL of water, the mixture was extracted with 2 x 50 mL of ethyl acetate. The combined organic extracts were washed with 20 mL of saturated sodium bicarbonate solution, brine, dried over sodium sulfate and

filtered. The filtrate was concentrated and the crude purified on a silica gel column with a 10:1 solvent mixture of hexane and ethyl acetate to yield 250 mg of 2-benzyl-5-tert-butyl-2H-pyrazole-3-carboxylic acid (4-trifluoromethyl-phenyl)-amide as an off-white solid.

Compounds AP-002 to AP-029:

These compounds were prepared according to the general procedure with the corresponding amine.

Compound AP-030:

A mixture of 1 g of ethyl 1-(4-methylbenzyl)-3-methylpyrazole-5-carboxylate (available from Maybridge Chemical Co., UK) in 5 mL each of ethanol and 2 N potassium hydroxide solution was heated at 90 °C for 4 hrs. The cooled reaction mixture was acidified with 6 N hydrochloric acid until pH 3. The resulting white solid was filtered, washed with water and chilled ethanol to yield 850 mg of 1-(4-methylphenyl)-3-methylpyrazole-5-carboxylic acid. To a solution of 226 mg of 1-(4-methylphenyl)-3-methylpyrazole-5-carboxylic acid in 3 mL of dimethylformamide was added 250 mg of 1,3-dicyclohexylcarbodiimide followed by 160 mg of 1-hydroxybenzotriazole. The reaction mixture was stirred at room temperature for 30 minutes, added with 130 mg of 4-triflurormethylaniline followed by 0.6 mL of diisopropylethylamine. The mixture was heated at 50 °C for 6 hrs, diluted with 50 mL of water and extracted with ethyl acetate. The organic extracts were washed with water, saturated sodium bicarbonate solution, brine, dried over sodium sulfate and filtered. Purification of the crude on silica gel column product 100 mg of 5-methyl-2-(4-methyl-benzyl)-2H-pyrazole-3-carboxylic acid (4-trifluoromethyl-phenyl)-amide as an off-white waxy solid.

Compound AP-031:

To a solution of 226 mg of 1-(4-methylbenzyl)-3-methylpyrazole-5-carboxylic acid in 3 mL of dimethylformamide was added 250 mg of 1,3-dicyclohexylcarbodiimide followed by 160 mg of 1-hydroxybenzotriazole. The reaction mixture was stirred at room temperature for 30 minutes, added with 130 mg of 3-triflurormethylaniline followed by 0.6 mL of diisopropylethylamine. The mixture was heated at 50 °C for 6 hrs, diluted with 50 mL of water and extracted with ethyl acetate. The organic extracts were washed with

water, saturated sodium bicarbonate solution, brine, dried over sodium sulfate and filtered. Purification of the crude on silica gel column produced 95 mg of 5-methyl-2-(4-methyl-benzyl)-2H-pyrazole-3-carboxylic acid (3-trifluoromethyl-phenyl)-amide as an off-white waxy solid.

Compound AP-032:

A mixture of 1 g of ethyl 1-(4-chlorobenzyl)-3-methylpyrazole-5-carboxylate (available from Maybridge Chemical Co., UK) in 5 mL each of ethanol and 2 N potassium hydroxide solution was heated at 90 °C for 4 hrs. The cooled reaction mixture was acidified with 6N hydrochloric acid until pH 3. The resulting white solid was filtered, washed with water and chilled ethanol to yield 900 mg of 1-(4-chlorophenyl)-3-methylpyrazole-5-carboxylic acid. To a solution of 226 mg of 1-(4-chlorolphenyl)-3-methylpyrazole-5-carboxylic acid in 3 mL of dimethylformamide was added 250 mg of 1,3-dicyclohexylcarbodiimide followed by 160 mg of 1-hydroxybenzotriazole. The reaction mixture was stirred at room temperature for 30 minutes, added with 130 mg of 4-triflurormethylaniline and 0.6 mL of diisopropylethylamine. The mixture was heated at 50 °C for 6 hrs, diluted with 50 mL of water and extracted with ethyl acetate. The organic extracts were washed with water, saturated sodium bicarbonate solution, brine, dried over sodium sulfate and filtered. Purification of the crude on silica gel column produced 110 mg of 5-methyl-2-(4-chloro-benzyl)-2H-pyrazole-3-carboxylic acid (4-trifluoromethyl-phenyl)-amide as a waxy solid.

Compound AP-033:

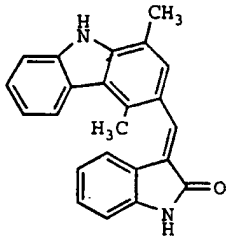
To a solution of 226 mg of 1-(4-chlorobenzyl)-3-methylpyrazole-5-carboxylic acid in 3 mL of dimethylformamide was added 250 mg of 1,3-dicyclohexylcarbodiimide followed by 160 mg of 1-hydroxybenzotriazole. The reaction mixture was stirred at room temperature for 30 minutes, added with 130 mg of 3-triflurormethylaniline and 0.6 mL of diisopropylethylamine. The mixture was heated at 50 °C for 6 hrs, diluted with 50 mL of water and extracted with ethyl acetate. The organic extracts were washed with water, saturated sodium bicarbonate solution, brine, dried over sodium sulfate and filtered. Purification of the crude on silica gel column produced 102 mg of 5-methyl-2-(4-chloro-benzyl)-2H-pyrazole-3-carboxylic acid (3-trifluoromethyl-phenyl)-amide as a waxy solid.

EXAMPLE 3: PROCEDURES FOR SYNTHESIZING THE INDOLINONE COMPOUNDS OF THE INVENTION

I. Synthesis of 2-Indolinones

A reaction mixture of the proper oxindole (1.0 equiv.), the appropriate aldehyde (1.2 equiv.), and piperidine (0.1 equiv.) in ethanol (1-2 mL/1.0 mmol oxindole) was stirred at 90 °C for 3-5 hours. After cooling, the precipitate was filtered, washed with cold ethanol, and dried to yield the target compound.

The compound of formula XXIV was synthesized using the above procedure and was characterized using ^{1}H NMR spectroscopy.



(XXIV)

NMR data is as follows: ^{1}H NMR (360 MHz, DMSO-d6) 11.46 (s, br, 1H NH-9'), 10.52 (s, br, 1H NH-1), 8.19 (d, *J*=8.38 Hz, 1H, H-5'), 7.94 (s, 1H, H-vinyl), 7.58 (d, *J*=7.80 Hz, 1H, H-4), 7.46 (s, 1H, H-2'), 7.42 (dt, *J*=0.97, 7.44 Hz, 1H, H-6), 7.24 (d, *J*=7.91 Hz, 1H, H-8'), 7.14-7.26 (m, H-6' to H-8'), 6.86 (d, *J*=7.44 Hz, 1H, H-7), 6.75 (dt, *J*=7.80 Hz, 1H, H-5), 2.78 (s, 3H, CH_3-1'(4')), 2.55 (s, 3H, CH_3-4'(1')).

II. Synthesis of Oxindoles and Aldehydes

All of the oxindoles and aldehydes used for preparing the examples in this patent are either commercially available or prepared by the methods described below.

A. Synthesis of Oxindoles

5-Amino-2-oxindole (O-1)

5-Nitro-2-oxindole (6.3 g) was hydrogenated in methanol over 10% palladium on carbon to give 3.0 g (60% yield) of the title compound as a white solid.

5-Bromo-2-oxindole (O-2)

2-Oxindole (1.3 g) in 20 mL of acetonitrile was cooled to -10 °C and 2.0 g of N-bromosuccinimide was slowly added with stirring. The reaction was stirred for 1 hour at -10 °C and 2 hours at 0 °C. The precipitate was collected, washed with water and dried to give 1.9 g (90% yield) of the title compound.

5-Chloro-2-oxindole (O-3)

5-Chloro-2-oxindole is commercially available from Aldrich Chemicals.

5,6-Dimethoxy-2-oxindole (O-4)

5,6-Dimethoxy-2-oxindole is commercially available from Maybridge.

2-Oxindole (O-5)

2-Oxindole is commercially available from Aldrich Chemicals.

4-Methyl-2-oxindole (O-6)

Diethyl oxalate (30 mL) in 20 mL of dry ether was added with stirring to 19 g of potassium ethoxide suspended in 50 mL of dry ether. The mixture was cooled in an ice bath and 20 mL of 3-nitro-o-xylene in 20 mL of dry ether was slowly added. The thick dark red mixture was heated to reflux for 0.5 hr, concentrated to a dark red solid, and treated with 10% sodium hydroxide until almost all of the solid dissolved. The dark red mixture was treated with 30% hydrogen peroxide until the red color changed to yellow. The mixture was treated alternately with 10% sodium hydroxide and 30% hydrogen peroxide until the dark red color was no longer present. The solid was filtered off and the filtrate acidified with 6 N hydrochloric acid. The resulting precipitate was collected by vacuum filtration, washed with water, and dried under vacuum to give 9.8 g (45% yield) of 1-methyl-6-nitrophenylacetic acid as an off-white solid. The solid was hydrogenated in methanol over 10% palladium on carbon to give 9.04 g of the title compound as a white solid.

5,7-Dibromo-2-oxindole (O-7)

Same as the preparation procedures for 5-bromo-2-oxindole (O-2), using 2 equivalents of N-bromosuccinimide.

7-Bromo-5-chloro-2-oxindole (O-8)

5-Chloro-2-oxindole (16.8 g) and 19.6 g of N-bromosuccinimide were suspended in 140 mL of acetonitrile and was heated to reflux for 3 hours. Thin layer chromatography (silica, ethyl acetate) at 2 hours of reflux showed 5-chloro-2-oxindole or N-bromosuccinimide (Rf 0.8), product (Rf 0.85) and a second product (Rf 0.9) whose proportions did not change after another hour of reflux. The mixture was cooled to 10 °C and the precipitate collected by vacuum filtration, washed with 25 mL of ethanol and sucked dry for 20 minutes in the funnel to give 14.1 g of wet product (56% yield). The solid was suspended in 200 mL of denatured ethanol and slurry-washed by stirring and heating to reflux for 10 minutes. The mixture was cooled in an ice bath to 10 °C. The solid product was collected by vacuum filtration, washed with 25 mL of ethanol and dried under vacuum at 44 °C to give 12.7 g (51% yield) of 7-bromo-5-chloro-2-oxindole.

5-Fluoro-2-oxindole (O-9)

5-Fluoroisatin (8.2 g) was dissolved in 50 mL of hydrazine hydrate and was heated to refulux for 1 hr. The reaction mixtures were then poured in ice water. The precipitate was then filtered, washed with water and dried under vacuum oven to give the title compound.

5-Nitro-2-oxindole (O-10)

2-Oxindole (6.5 g) was dissolved in 25 mL of concentrated sulfuric acid and the mixture maintained at -10 - 15 °C while 2.1 mL of fuming nitric acid was added dropwise. After the addition of the nitric acid the reaction mixture was stirred at 0 °C for 0.5 hr and poured into ice-water. The precipitate was collected by filtration, washed with water and crystallized from 50% acetic acid. The final crystalline product was then filtered, washed with water and dried under vacuum to give 6.3 g (70%) of 5-nitro-2-oxindole.

5-Iodo-2-oxindole (O-11)

2-Oxindole (82.9 g) was suspended in 630 mL of acetic acid with mechanical stirring and the mixture cooled to 10 °C in an ice water bath. Solid N-iodosuccinimide (175 g) was added in portions over 10 minutes. After the addition was complete the

mixture was stirred for 1 hour at 10 °C. The suspended solid which was always present became very thick at this time. The solid was collected by vacuum filtration, washed with 100 mL of 50% acetic acid in water and then with 200 mL of water and sucked dry for 20 minutes in the funnel. The product was dried under vacuum to give 93.5 g (36%) of 5-iodo-2-oxindole.

5-Methyl-2-oxindole (O-12)

5-Methylisatin (15.0 g) and 60 mL of hydrazine hydrate were heated to 140 - 160 °C for 4 hours. Thin layer chromatography (ethyl acetate:hexane 1:2, silica gel) showed no starting material remaining. The reaction mixture was cooled to room temperature, poured into 300 mL of ice water and acidified to pH 2 with 6 N hydrochloric acid. After standing at room temperature for 2 days the precipitate was collected by vacuum filtration, washed with water and dried under vacuum to give 6.5 g (47% yield) of 5-methyl-2-oxindole.

5-Bromo-4-methyl-2-oxindole (O-13) and 5,7-Dibromo-4-methyl-2-oxindole (O-32)

4-Methyl-2-oxindole (5 g) in 40 mL of acetonitrile was treated with 7.26 g of *N*-bromosuccinimide and stirred at room temperature for 4 hours. Thin layer chromatography (ethyl acetate:hexane 1:2, silica gel) showed a mixture of 5-bromo (Rf 0.3) and 5,7-dibromo (Rf 0.5) products. Another 7.26 g of N-bromosuccinimide was added and the mixture stirred for 4 additional hours. The solid was collected by vacuum filtration, washed with 20 mL of acetonitrile and dried to give a 1:1 mixture of mono and dibromo compounds. The filtrate was concentrated and chromatographed on silica gel (ethyl acetate:hexane 1:2) to give 1.67 g of 5-bromo-4-methyl-2-oxindole as a beige solid. The 1:1 mixture of solids was recrystallized twice from glacial acetic acid to give 3.2 g of 5,7-dibromo-4-methyl-2-oxindole as a light orange solid. The filtrates from this material were chromatographed as above to give 0.6 g of 5-bromo-4-methyl-2-oxindole and 0.5 g of 5,7-dibromo-4-methyl-2-oxindole.

6-Fluoro-2-oxindole (O-14)

Sodium hydride (2.6 g) and 14.5 g of dimethylmalonate was stirred and heated to 100 °C in 160 mL of dimethylsulfoxide for 1 hour. The mixture was cooled to room

temperature and 7.95 g of 2,5-difluoronitrobenzene added and stirred for 30 minutes. The mixture was then heated to 100 °C for 1 hour, cooled to room temperature and poured into 400 mL of saturated ammonium chloride solution. The mixture was extracted with 200 mL of ethyl acetate and the organic layer washed with brine, dried over anhydrous sodium sulfate and concentrated under vacuum. The residue was crystallized from methanol to give 24.4 g (80% yield) of dimethyl 4-fluoro-2-nitrophenylmalonate as a white solid. Thin layer chromatography (ethyl acetate:hexane 1:6, silica gel) Rf 0.2. The filtrate was concentrated and chromatographed on a column of silica gel (ethyl acetate:hexane 1:8) to give 5.03 g of dimethyl 4-fluoro-2-nitrophenylmalonate, for a total of 29.5 g (96% yield).

Dimethyl 4-fluoro-2-nitrophenylmalonate (5.0 g) was heated to reflux in 20 mL of 6 N hydrochloric acid for 24 hours. The reaction was cooled and the white solid collected by vacuum filtration, washed with water and dried to give 3.3 g (87% yield) of 4-fluoro-2-nitrophenylacetic acid. Thin layer chromatography (ethyl acetate:hexane 1:2, silica gel) Rf 0.6.

4-Fluoro-2-nitrophenylacetatic acid (3.3 g) dissolved in 15 mL of acetic acid was hydrogenated over 0.45 g of 10% palladium on carbon under 60 psi for 2 hours. The catalyst was removed by filtration and washed with 15 mL of methanol. The combined filtrates were concentrated and diluted with water. The precipitate was collected by vacuum filtration, washed with water and dried to give 1.6 g (70% yield) of 6-fluoro-2-oxindole. Thin layer chromatography Rf 0.24. The filtrate was concentrated to give a purple solid with an NMR spectrum similar to the first crop. Chromatography of the purple solid (ethyl acetate:hexane 1:2, silica gel) gave a second crop of 6-fluoro-2-oxindole as a white solid.

6-Chloro-2-oxindole (O-15)

6-Chlorooxindole is commercially available from Finorga.

5-Carboxyethyl-4-methyl-2-oxindole (O-16)

Same as the procedures for 5-Carboxyethyl-2-oxindole (O-27).

5-Aminosulfonyl-2-oxindole (O-17)

To a 100 mL flask charged with 27 mL of chlorosulfonic acid was added slowly

13.3 g of 2-oxindole. The reaction temperature was maintained below 30 °C during the addition. After the addition, the reaction mixture was stirred at room temperature for 1.5 hr, heated to 68 °C for 1 hr, cooled, and poured into water. The precipitate was washed with water and dried in a vacuum oven to give 11.0 g of 5-chlorosulfonyl-2-oxindole (50% yield) which was used without further purification.

5-Chlorosulfonyl-2-oxindole (2.1 g) was added to 10 mL of ammonium hydroxide in 10 mL of ethanol and stirred at room temperature overnight. The mixture was concentrated and the solid collected by vacuum filtration to give 0.4 g (20% yield) of the title compound as an off-white solid.

5-Methylaminosulfonyl-2-oxindole (O-18)

A suspension of 3.38 g of 5-chlorosulfonyl-2-oxindole from the oxindole O-17 in 10 mL of 2 M methylamine in tetrahydrofuran was stirred at room temperature for 4 hours at which time a white solid was present. The precipitate was collected by vacuum filtration, washed twice with 5 mL of water each time and dried under vacuum at 40 °C overnight to give 3.0 g (88% yield) of 5-methylaminosulfonyl-2-oxindole.

5-(4-Trifluoromethylphenylaminosulfonyl)-2-oxindole (O-19)

A suspension of 2.1 g of 5-chlorosulfonyl-2-oxindole from example O-18, 1.6 g of 4-trifluoromethylaniline and 1.4 g of pyridine in 20 mL of dichloromethane was stirred at room temperature for 4 hours. The precipitate was collected by vacuum filtration, washed twice with 5 mL of water each time and dried under vacuum at 40 °C overnight to give 2.4 g of crude product containing some impurities by thin layer chromatography. The crude product was chromatographed on silica gel eluting with ethyl acetate:hexane 1:2 to give 1.2 g (37% yield) of 5-(4-trifluoromethylphenylaminosulfonyl)-2-oxindole.

5-(Morpholin-4-sulfonyl)-2-oxindole (O-20)

A suspension of 2.3 g of 5-chlorosulfonyl-2-oxindole from example O-18 and 2.2 g of morpholine in 50 mL of dichloromethane was stirred at room temperature for 3 hours. The white precipitate was collected by vacuum filtration, washed with ethyl acetate and hexane and dried under vacuum at 40 °C overnight to give 2.1 g (74% yield) of 5-(morpholin-4-sulfonyl)-2-oxindole.

6-Trifluoromethyl-2-oxindole (O-21)

Dimethylsulfoxide (330 mL) was added to 7.9 g of sodium hydride followed by, dropwise, 43.6 g of diethyloxalate. The mixture was heated to 100 °C for 1 hour and cooled to room temperature. 2-Nitro-4-trifluromethyltoluene (31.3 g) was added, the reaction stirred for 30 minutes at room temperature and then heated to 100 °C for 1 hour. The reaction was cooled and poured into a mixture of saturated aqueous ammonium chloride, ethyl acetate and hexane. The organic layer was washed with saturated ammonium chloride, water and brine, dried, and concentrated to give dimethyl 2-(2-nitro-4-trifluoromethylphenyl)malonate.

The diester was dissolved in a mixture of 6.4 g of lithium chloride and 2.7 mL of water in 100 mL of dimethylsulfoxide and heated to 100 °C for 3 hours. The reaction was cooled and poured into a mixture of ethyl acetate and brine. The organic phase was washed with brine, dried with sodium sulfate, concentrated and chromatographed on silica gel in 10% ethyl acetate in hexane. The fractions containing product were evaporated to give 25.7 g of methyl 2-nitro-4-trifluoromethylphenylacetate.

Methyl 2-nitro-4-trifluoromethylphenylacetate (26 mg) was hydrogenated over 10% palladium on carbon and then heated at 100 °C for 3 hours. The catalyst was removed by filtration and the solvent evaporated to give the title compound.

5-(2-Chloroethyl)-2-oxindole (O-22)

5-Chloroacetyl-2-oxindole (prepared by the same procedure used for the preparation of O-53, except starting from chloroacetyl chloride) (4.18 g) in 30 mL of trifluoroacetic acid in an ice bath was treated with 4.65 g of triethylsilane and stirred at room temperature for 3 hours. The mixture was poured into 150 mL of water and the precipitate collected by vacuum filtration, washed with 50 mL of water and dried to give 2.53 g (65% yield) of 5-(2-chloroethyl)-2-oxindole as a reddish-brown solid.

4-Methoxycarbonyl-2-oxindole (O-23)

Esterification of 4-carboxy-2-oxindole (O-25) with trimethylsilyldiazomethane.

5-Methoxycarbonyl-2-oxindole (O-24)

5-Iodo-2-oxindole (17 g) was was heated to refulux with 2 g of palladium diacetate, 18.2 g of triethylamine, 150 mL of methanol, 15 mL of dimethylsulfoxide and 2.6 g of DPPP in an atmosphere saturated with carbon monoxide. After 24 hours, the reaction was filtered to remove the catalyst and the filtrate concentrated. The concentrate was chromatographed on silica gel in 30% ethyl acetate in hexane. The fractions containing product were concentrated and allowed to stand. The precipitated product was collected by vacuum filtration to give 0.8 g (7%) of the title compound as an off-white solid.

4-Carboxy-2-oxindole (O-25)

A solution of trimethylsilyldiazomethane in hexane (2 M)was added dropwise to the solution of 2.01 g of 2-chloro-3-carboxy-nitrobenzene in 20 mL of methanol at room temperature until no gas evolution occurred. The excess trimethylsilyldiazomethane was quenched with acetic acid. The reaction mixture was dried by rotary pump and the residue was further dried in oven for overnight. The product (2-chloro-3-methoxycarbonyl-nitrobenzene) was pure enough for the following reaction.

Dimethyl malonate (6.0 mL) was added to the ice-cold suspension of 2.1 g of sodium hydride in 15 mL of DMSO. The reaction mixture was then stirred at 100 °C for 1 h and cooled to room temperature. 2-Chloro-3-methoxycarbonyl-nitrobenzene (2.15 g) was added to the above mixture in one portion and the mixture was heated to 100 °C for 1.5 h. The reaction mixture was then cooled to room temperature and poured into ice water, acidified to pH of 5, and extracted with ethyl acetate. The organic layer was then washed with brine, dried over anhydrous sodium sulfate and concentrated to give 3.0 g of the dimethyl 2-methoxycarbonyl-6-nitrophenylmalonate.

Dimethyl 2-methoxycarbonyl-6-nitrophenylmalonate (3.0 g) was was heated to refulux in 50 mL of 6 N hydrochloric acid overnight. The mixture was concentrated to dryness and was heated to refulux for 2 hours with 1.1 g of tin(II) chloride in 20 mL of ethanol. The mixture was filtered through Celite, concentrated and chromatographed on silica gel in ethyl acetate:hexane:acetic acid to give 0.65 g (37% yield) of 4-carboxy-2-oxindole as a white solid.

5-Carboxy-2-oxindole (O-26)

2-Oxindole (6.7 g) was added to a stirred suspension of 23 g of aluminum chloride in 30 mL of dichloroethane in an ice bath. Chloroacetyl chloride (11.3 g) was slowly added and hydrogen chloride gas was evolved. After ten minutes of stirring, the reaction was warmed to 40 - 50 °C for 1.5 hours. Thin layer chromatography (ethyl acetate, silica gel) showed no remaining starting material. The mixture was cooled to room temperature and poured into ice water. The precipitate was collected by vacuum filtration, washed with water and dried under vacuum to give 10.3 g (98%) of 5-chloroacetyl-2-oxindole as an off-white solid.

A suspension of 9.3 g of 5-chloroacetyl-2-oxindole was stirred in 90 mL pyridine at 80 - 90 °C for 3 hours then cooled to room temperature. The precipitate was collected by vacuum filtration and washed with 20 mL of ethanol. The solid was dissolved in 90 mL of 2.5 N sodium hydroxide and stirred at 70 - 80 °C for 3 hours. The mixture was cooled to room temperature and acidified to pH 2 with 0.5 N hydrochloric acid. The precipitate was collected by vacuum filtration and washed thoroughly with water to give crude 5-carboxy-2-oxindole as a dark brown solid. After standing overnight the filtrate yielded 2 g of 5-carboxy-2-oxindole as a yellow solid. The crude dark brown product was dissolved in hot methanol, the insoluble material removed by filtration and the filtrate concentrated to give 5.6 g of 5-carboxy-2-oxindole as a brown solid . The combined yield was 97%.

5-Carboxyethyl-2-oxindole (O-27)

5-Cyanoethyl-2-oxindole (4.02 g) in 10 mL of water containing 25 mL of concentrated hydrochloric acid was was heated to refulux for 4 hours. The mixture was cooled, water added and the resulting solid collected by vacuum filtration, washed with water and dried to give 1.9 g (44% yield) of the title compound as a yellow solid.

5-Amino-4-methyl-2-oxindole (O-28)

Same as the procedures for 5-aminooxindole (O-1).

5-Nitro-4-methyl-2-oxindole (O-29)

Same as the procedures for 5-nitrooxindole (O-10).

5-Chloro-4-methyl-2-oxindole (O-30)

A suspension of 3.0 g of 4-methyl-2-oxindole was stirred in 50 mL of acetonitrile at room temperature while 3.3 g of N-chlorosuccinimide was added in portions. Trifluoroacetic acid (1 mL) was then added. The suspension was stirred at room temperature for 3 days during which time solid was always present. The white solid was collected by vacuum filtration, washed with a small amount of cold acetone and dried overnight in a vacuum oven at 40 °C to give 2.5 g (68%) of 5-chloro-4-methyl-2-oxindole.

5-Iodo-4-methyl-2-oxindole (O-31)

To 2 g of 4-methyl-2-oxindole in 40 mL of glacial acetic acid in an ice bath was added 3.67 g of N-iodosuccinimide. The mixture was stirred for 1 hour, diluted with 100 mL of 50% acetic acid in water and filtered. The resulting white solid was dried under high vacuum to give 3.27 g (88% yield) of the title compound as an off-white solid.

5,7-Dibromo-4-methyloxindole (O-32)

See procedures for 5-bromo-4-methyloxindole (O-13).

5-Butyl-2-oxindole (O-33)

Triethylsilane (2.3 g) was added to 2 g of 4-butanoyl-2-oxindole in 20 mL of trifluoroacetic acid at room temperature and the solution stirred for 3 hours. The reaction was poured into ice water to give a red oil which solidified after standing. The solid was collected by vacuum filtration, washed with water and hexane and dried to give 1.7 g (91% yield) of the title compound as an off-white solid.

5-Ethyl-2-oxindole (O-34)

5-Acetyl-2-oxindole (2 g) in 15 mL of trifluoroacetic acid in an ice bath was slowly treated with 1.8 g of triethylsilane and then stirred at room temperature for 5 hours. One mL of triethylsilane was added and the stirring continued overnight. The reaction mixture was poured into ice water and the resulting precipitate collected by vacuum filtration, washed copiously with water and dried under vacuum to give 1.3 g (71% yield) of the title compound as a yellow solid.

5-(Morpholin-4-yl)ethyl-2-oxindole (O-35)

5-Chloroethyl-2-oxindole (2.3 g), 1.2 mL of morpholine and 1.2 mL of diisopropylethylamine were heated overnight at 100 °C in 10 mL of dimethylsulfoxide. The mixture ws cooled, poured into water and extacted with ethyl acetate. The organic layer was washed with brine, dried and evaporated. The residue was chromatographed on silica gel in 5% methanol in chloroform to give 0.9 g (31%) of the title compound as a white solid.

5-(4-Carboxybenzamido)-2-oxindole (O-36)

5-(4-Methoxycarbonylbenzamido)-2-oxindole (0.9 g) and 0.4 g of sodium hydroxide in 25 mL of methanol was heated to refulux for 3 hours. The mixture was concentrated, water added, and the mixture acidified with 6 N hydrochloric acid. The precipitate was collected by vacuum filtration to give 0.75 g (87%) of the title compound as a white solid.

5-(4-Methoxycarbonylbenzamido)-2-oxindole (O-37)

A mixture of 82.0 mg of 5-amino-2-oxindole and 131.0 mg of 4-methoxycarbonylbenzoyl chloride in pyridine was stirred at room temperature for 3 hr and poured into ice water. The precipitate was filtered, washed with water and dried in a vacuum oven to give 138.0 mg of 5-(4-methoxycarbonylbenzamido)-2-oxindole (81% yield).

5-Methoxy-2-oxindole (O-38)

Chloral hydrate (9.6 g) was dissolved in 200 mL of water containing 83 g of sodium sulfate. The solution was warmed to 60 °C, a solution of 11.4 g of hydroxylamine hydrochloride in 50 mL of water was added and the mixture was held at 60 °C. In a separate flask , 6.4 g of 4-anisidine and 4.3 mL of concentrated hydrochloric acid in 80 mL of water was warmed to 80 °C. The first solution was added to the second and was heated to refulux. The reaction was heated to refulux for 2 minutes, cooled slowly to room temperature, and then cooled in an ice bath. The tan precipitate was collected by vacuum filtration, washed with water and dried under vacuum to give 8.6 g (85% yield) of

N-(2-hydroximinoacetyl)anisidine.

Concentrated sulfuric acid (45 mL) containing 5 mL of water was warmed to 60 °C and 8.6 g of N-(2-hydroximinoacetyl)anisidine was added in one portion. The stirred mixture was heated to 93 °C for 10 minutes and then allowed to cool to room temperature. The mixture was poured into 500 g of ice and extracted 3 times with ethyl acetate. The combined extracts were dried over anhydrous sodium sulfate and concentrated to give 5.1 g (65% yield) of 5-methoxyisatin as a dark red solid.

5-Methoxyisatin (5.0 g) and 30 mL of hydrazine hydrate were heated to reflux for 15 minutes. The reaction mixture was cooled to room temperature and 50 mL of water was added. The mixture was extracted 3 times with 25 mL of ethyl acetate each time, the organic layers combined, dried over anhydrous sodium sulfate and concentrated to give a yellow solid. The solid was stirred in ethyl acetate and 1.1 g of insoluble material removed by vacuum filtration and saved. This material proved to be 2-hydrazinocarbonylmethyl-4-anisidine. The filtrate was concentrated and chromatographed on silica gel eluting with ethyl acetate:hexane 1:1 to give 0.7 g of 5-methoxy-2-oxindole as a dirty yellow solid. The 1.1 g of 2-hydrazinocarbonylmethyl-4-anisidine was heated to refulux for 1 hour in 20 mL of 1 N sodium hydroxide. The mixture was cooled, acidified to pH 2 with concentrated hydrochloric acid and extracted 3 times with 25 mL of ethyl acetate each time. The organic extracts were combined, washed with brine, dried over anhydrous sodium sulfate and concentrated to give 0.8 g of 5-methoxy-2-oxindole as a yellow solid. The combined yield was 1.5 g or 33%.

Reference:

Crestin, C., and R. Saladino, Synthetic Communications 24:2839-2841 (1994).

7-Aza-2-oxindole (O-39)

3,3-Dibromo-7-azaoxindole (2.9 g) was dissolved in a mixture of 20 mL of acetic acid and 30 mL of acetonitrile. To the solution was added 6.5 g of zinc dust. The mixture was stirred for 2 hrs at room temperature. The solid was filtered from the mixture and the solvent evaporated. The residue was treated with ethyl acetate. The ethyl acetate solution containing insoluble solid was passed through a short column of silica gel. The collected ethyl acetate solution was evaporated and the residue dried under vacuum to give 1.8 g

(yield 91%) of 7-azaoxindole acetic acid salt.

5-Chloro-7-methyl-2-oxindole (O-40)

Same procedures for 5-fluorooxindole (O-9).

7-Fluoro-2-oxindole (O-41)

Same as the procedure for 6-fluorooxindole (O-14).

4-Fluoro-2-oxindole (O-42)

Same as the procedure for 6-Fluorooxindole (O-14).

6-Methoxy-2-oxindole (O-43)

6-Methoxyoxindole is commercially available from Finorga.

5-Trifluoromethyl-2-oxindole (O-44)

Same procedures for 5-fluorooxindole (O-9).

5-Dimethylaminosulfonyl-2-oxindole (O-45)

A suspension of 2.3 g of 5-chlorosulfonyl-2-oxindole (O-17) in 10 mL of 2 M dimethylamine in methanol was stirred at room temperature for 4 hours at which time a white solid was present. The precipitate was collected by vacuum filtration, washed with 5 mL of 1 N sodium hydroxide and 5 mL of 1 N hydrochloric acid and dried under vacuum at 40 °C overnight to give 1.9 g (79% yield) of 5-dimethylaminosulfonyl-2-oxindole.

6-Phenyl-2-oxindole (O-46)

Dimethyl malonate (10 mL) in 25 mL of dimethylsulfoxide was added dropwise to 3.5 g of sodium hydride suspended in 25 mL of dimethylsulfoxide and the mixture heated at 100 °C for 10 minutes. The mixture was cooled to room temperature and 4.7 g of 4-fluoro-3-nitrobiphenyl in 25 mL of dimethylsulfoxide was added. The mixture was heated at 100 °C for 2 hours, cooled and quenched with 300 mL of saturated ammonium chloride solution. The mixture was extracted three times with ethyl acetate and the combined

organic layers washed with water and brine and evaporated to give a yellow oil, crude dimethyl-3-nitrobiphenyl-4-malonate.

Crude dimethyl-3-nitrobiphenyl-4-malonate was heated to refulux in 30 mL of 6 N hydrochloric acid for 24 hours. The precipitate was collected by filtration, washed with water and dried to give 4.5 g (80% based on 4-fluoro-3-nitrobiphenyl) of 3-nitrobiphenyl-4-acetic acid as a cream colored solid.

Iron powder (2.6 g) was added all at once to 4.5 g of 3-nitrobiphenyl-4-acetic acid in 40 mL of acetic acid. The mixture was heated to refulux for 2 hours, concentrated to dryness and taken up in ethyl acetate. The solids were removed by filtration and the filtrate washed twice with 1 N hydrochloric acid and brine and dried over anhydrous sodium sulfate. The filtrate was concentrated to give 3.4 g (93% yield) of 6-phenyl-2-oxindole as a light brown solid.

6-(2-Methoxyphenyl)-2-oxindole (O-47)

Tetrakis(triphenylphosphine)palladium (1 g) was added to a mixture of 5 g of 2-methoxyphenylboronic acid, 6.6 g of 5-bromo-2-fluoronitrobenzene and 30 mL of 2 M sodium carbonate solution in 50 mL of toluene and 50 mL of ethanol. The mixture was heated to refulux for 2 hours, concentrated, and the residue extracted twice with ethyl acetate. The ethyl acetate layer was washed with water, brine, dried, and concentrated to give a dark green oil which solidified on standing, crude 4-fluoro-2'-methoxy-3-nitrobiphenyl.

Dimethyl malonate (14 mL) was added dropwise to 2.9 g of sodium hydride suspended in 50 mL of dimethylsulfoxide. The mixture was heated at 100 °C for 15 minutes and cooled to room temperature. Crude 4-fluoro-2'-methoxy-3-nitrobiphenyl in 60 mL of dimethylsulfoxide was added and the mixture was heated at 100 °C for 2 hours. The reaction mixture was cooled and quenched with 300 mL of saturated sodium chloride solution and extracted twice with ethyl acetate. The extracts were combined, washed with saturated ammonium chloride, water, and brine, dried over anhydrous sodium sulfate and concentrated to give crude dimethyl 2'-methoxy-3-nitrobiphenyl-4-malonate as a yellow oil.

Crude 2'-methoxy-3-nitrobiphenyl-4-malonate was heated at 100 °C in 50 mL of 6 N hydrochloric acid for 24 hours and cooled. The precipitate was collected by filtration,

washed with water and hexane, and dried to give 9.8 of 2'-methoxy-2-nitrobiphenyl-4-acetic acid as a light tan solid.

Iron powder (5 g) was added in one portion to 9.8 g of 2'-methoxy-3-nitrobiphenyl-4-acetic acid in 50 mL of glacial acetic acid was heated to 100 °C for 3 hours. The reaction mixture was concentrated to dryness, sonicated in ethyl acetate and filtered to remove the insolubles. The filtrate was washed twice with 1 N hydrochloric acid, water, brine, dried over anhydrous sodium sulfate and concentrated. The residue was chromatographed on silica gel in ethyl acetate:hexane 1:2 to give 5.4 g (69% yield based on 5-bromo-2-fluoronitrobenzene) of 6-(2-methoxyphenyl)-2-oxindole as a rose colored solid.

6-(3-Methoxyphenyl)-2-oxindole (O-48)

Tetrakis(triphenylphosphine)palladium (0.8 g) was added to a mixture of 5 g of 3-methoxyphenylboronic acid, 5 g of 5-bromo-2-fluoronitrobenzene and 11 mL of 2 M sodium carbonate solution in 100 mL of toluene. The mixture was heated to refulux for 2 hours, diluted with water and extracted with ethyl acetate. The ethyl acetate was washed with saturated sodium bicarbonate, brine, dried, and concentrated to give an oily solid. The solid was chromatographed on silica gel in ethyl acetate:hexane 1:6 to give 4.3 g (77% yield) of 4-fluoro-3'-methoxy-3-nitrobiphenyl.

Dimethyl malonate (9.7 mL) was added dropwise to 2.0 g of sodium hydride suspended in 50 mL of dimethylsulfoxide. The mixture was heated to 100 °C for 35 minutes and cooled to room temperature. 4-Fluoro-2'-methoxy-3-nitrobiphenyl (4.2 g) in 50 mL of dimethylsulfoxide was added and the mixture was heated at 100 °C for 1 hours. The reaction mixture was cooled and quenched with 300 mL of saturated ammonium chloride solution and extracted twice with ethyl acetate. The extracts were combined, washed with brine, dried over anhydrous sodium sulfate and concentrated to give crude dimethyl 3'-methoxy-3-nitrobiphenyl-4-malonate as a pale yellow solid.

Crude 3'-methoxy-3-nitro-biphenyl-4-malonate was heated at 110 °C in 45 mL of 6 N hydrochloric acid for 4 days and cooled. The precipitate was collected by filtration, washed with water and hexane, and dried to give 5.3 g of 3'-methoxy-2-nitrobiphenyl-4-acetic acid as a light tan solid.

3'-Methoxy-3-nitrobiphenyl-4-acetic acid (5.2 g) was dissolved in methanol and

hydrogenated over 0.8 g of 10% palladium on carbon for 3 hours at room temperature. The catalyst was removed by filtration, washed with methanol and the filtrates combined and concentrated to give a brown solid. The solid was chromatographed on silica gel in ethyl acetate:hexane:acetic acid 33:66:1 to give 3.0 g (75% yield based on 4-fluoro-3'-methoxy-3-nitrobiphenyl) of 6-(3-methoxypheny)-2-oxindole as a pink solid.

6-(4-Methoxyphenyl)-2-oxindole (O-49)

Tetrakis(triphenylphosphine)palladium (1 g) was added to a mixture of 5 g of 4-methoxyphenylboronic acid, 6.6 g of 5-bromo-2-fluoronitrobenzene and 30 mL of 2 M sodium carbonate solution in 50 mL of toluene and 50 mL of ethanol. The mixture was heated to refulux for 2 hours, concentrated, and the residue extracted twice with ethyl acetate. The ethyl acetate layer was washed with water, brine, dried, and concentrated to give a brown oily solid. The solid was chromatographed on silica gel in 5% ethyl acetate in hexane to give crude 4-fluoro-4'-methoxy-3-nitrobiphenyl as a pale yellow solid.

Dimethyl malonate (10 mL) was added dropwise to 2.0 g of sodium hydride suspended in 60 mL of dimethylsulfoxide. The mixture was heated to 100 °C for 10 minutes and cooled to room temperature. Crude 4-fluoro-2'-methoxy-3-nitrobiphenyl (5.2 g) in 50 mL of dimethylsulfoxide was added and the mixture was heated at 100 °C for 2 hours. The reaction mixture was cooled and quenched with 300 mL of saturated sodium chloride solution and extracted three times with ethyl acetate. The extracts were combined, washed with saturated ammonium chloride, water and brine, dried over anhydrous sodium sulfate and concentrated to give crude dimethyl 4'-methoxy-3-nitrobiphenyl-4-malonate as a yellow oil.

Crude 4'-methoxy-3-nitro-biphenyl-4-malonate was heated at 100 °C in 60 mL of 6 N hydrochloric acid for 15 hours and cooled. The precipitate was collected by filtration, washed with water and hexane, and dried to give 7.2 g of crude 4'-methoxy-3-nitrobiphenyl-4-acetic acid as a light tan solid.

Iron powder (3.6 g) was added in one portion to 7.2 g of 4'-methoxy-3-nitrobiphenyl-4-acetic acid in 50 mL of glacial acetic acid and heated at 100 °C overnight. The reaction mixture was concentrated to dryness, sonicated in ethyl acetate and filtered to remove the insolubles. The filtrate was washed twice with 1 N hydrochloric acid, brine, dried over anhydrous sodium sulfate and concentrated to give 2.7 g (54% yield based on

5-bromo-2-fluoronitrobenzene) of 6-(4-methoxyphenyl)-2-oxindole as a rose colored solid.

6-(3-Ethoxyphenyl)-2-oxindole (O-50)

Tetrakis(triphenylphosphine)palladium (0.8 g) was added to a mixture of 4.2 g of 3-ethoxyphenylboronic acid, 5.0 g of 5-bromo-2-fluoronitrobenzene and 22 mL of 2 M sodium carbonate solution in 50 mL of toluene and 50 mL of ethanol. The mixture was heated to refulux for 2 hours, concentrated, water was added and the mixture was extracted twice with ethyl acetate. The ethyl acetate layer was washed with water, brine, dried, and concentrated. The residue was chromatographed on silica gel in 5% ethyl acetate in hexane to give 5.3 g (90% yield) of crude 4-fluoro-3'-ethoxy-3-nitrobiphenyl as a yellow oil.

Dimethyl malonate (11.4 mL) was added dropwise to 4.0 g of sodium hydride suspended in 20 mL of dimethylsulfoxide. The mixture was heated to 100 °C for 10 minutes and cooled to room temperature. Crude 4-fluoro-3'-ethoxy-3-nitrobiphenyl (5.3 g) in 25 mL of dimethylsulfoxide was added and the mixture was heated at 100 °C for 2 hours. The reaction mixture was cooled and quenched with 300 mL of saturated amonium chloride solution and extracted three times with ethyl acetate. The extracts were combined, washed with water and brine, dried over anhydrous sodium sulfate and concentrated to give crude dimethyl 3'-ethoxy-3-nitrobiphenyl-4-malonate as a yellow oil.

Crude dimethyl 3'-ethoxy-3-nitrobiphenyl-4-malonate was heated at 100 °C in 60 mL of 6 N hydrochloric acid for a total of 4 days and cooled. The precipitate was collected by filtration, washed with water and hexane, and dried to give 4.7 g (77% yield based on 5-bromo-2-fluoronitrobenzene) of crude 3'-ethoxy-3-nitrobiphenyl-4-acetic acid as a light tan solid.

Iron powder (2.4 g) was added in one portion to 4.6 g of 3'-ethoxy-3-nitrobiphenyl-4-acetic acid in 40 mL of glacial acetic acid and was heated to refulux for 2 hours. The reaction mixture was concentrated to dryness, treated repeatedly with ethyl acetate and filtered to remove the insolubles. The filtrate was washed twice with 1 N hydrochloric acid, brine, dried over anhydrous sodium sulfate and concentrated to give 3.5 g (91% yield) of 6-(3-ethoxyphenyl)-2-oxindole as a light brown solid.

6-Bromo-2-oxindole (O-51)

Dimethyl malonate (13 mL) was added dropwise to 2.7 g of sodium hydride suspended in 20 mL of dimethylsulfoxide. The mixture was heated to 100 °C for 10 minutes and cooled to room temperature. 5-Bromo-2-fluoronitrobenzene (5.0 g) in 25 mL of dimethylsulfoxide was added and the mixture was heated at 100 °C for 2 hours. The reaction mixture was cooled and quenched with 300 mL of saturated ammonium chloride solution and extracted three times with ethyl acetate. The extracts were combined, washed with saturated ammonium chloride, water and brine, dried over anhydrous sodium sulfate and concentrated to give crude dimethyl 4-bromo-2-nitrophenylmalonate as a pale yellow oil.

Crude dimethyl 4-bromo-2-nitrophenylmalonate was heated at 110 °C in 40 mL of 6 N hydrochloric acid for 24 hours and cooled. The precipitate was collected by filtration, washed with water and dried to give 5.3 g (89% yield) of 4-bromo-2-nitrophenylacetic acid as an off white solid.

4-Bromo-2-nitrophenylacetic acid 0.26 g), 0.26 g of zinc powder and 3 mL of 50% sulfuric acid in 5 mL of ethanol was heated at 100 °C overnight. The reaction mixture was filtered, diluted with a little acetic acid, concentrated to remove ethanol, diluted with water and extracted twice with ethyl acetate. The combined extracts were washed with brine, dried over anhydrous sodium sulfate and concentrated to give 0.19 g (90% yield) of 6-bromo-2-oxindole as a yellow solid.

5-Carboxy-4-methyl-2-oxindole (O-52)

Same procedures as that for 5-carboxyoxindole (O-26).

5-Acetyl-2-oxindole (O-53)

2-Oxindole (3 g) was suspended in 1,2-dichloroethane and slowly treated with 3.2 mL of acetyl chloride. The resulting suspension was heated to 50 °C for 5 hours, cooled, and poured into water. The resulting precipitate was collected by vacuum filtration, washed copiously with water and dried under vacuum to give 2.9 g (73% yield) of the title compound as a brown solid.

5-Butanoyl-2-oxindole (O-54)

To 15 g of aluminum chloride suspended in 30 mL of 1,2-dichloroethane in an ice bath was added 7.5 g of 2-oxindole and then 12 g of butanoyl chloride. The resulting suspension was heated to 50 °C overnight. The mixture was poured into ice water and extracted 3 times with ethyl acetate. The combined ethyl acetate layers were washed with brine, dried over sodium sulfate, and concentrated to dryness to give a brown solid. The solid was chromatographed on silica gel in 50% ethyl acetate in hexane to give 3 g (25%) of the title compound as a yellow solid.

5-Cyanoethyl-2-oxindole (O-55)

Potassium cyanide (2.0 g) was added to 15 mL of dimethylsulfoxide and heated to 90 °C. 5-Chloroethyl-2-oxindole (3.0 g) dissolved in 5 mL of dimethyl sulfoxide was added slowly with stirring, and the reaction heated to 150 °C for 2 hours. The mixture was cooled, poured into ice water and the precipitate collected by vacuum filtration, washed with water, and dried to give crude product. The crude material was chromatographed on silica gel in 5% methanol in chloroform to give 1.2 g (42% yield) of the title compound.

4-Chloro-2-oxindole (O-56)

Same procedures as that for 5-fluorooxindole (O-9).

6-Amino-2-oxindole (O-57)

6-Aminooxindole was synthesized using the procedure set forth in Helv. Chem. Acta 31:1381, 1948.

6-(Morpholin-4-yl)-2-oxindole (O-58)

6-Amino-2-oxindole (2.2 g), 4.0 g of 2,2'-dibromoethyl ether and 7.9 g of sodium carbonate were refluxed in 20 mL of ethanol overnight, concentrated and diluted with 50 mL of water. The mixture was extracted three times with 50 mL of ethyl acetate each time and the organic extracts combined, washed with 20 mL of brine, dried over anhydrous sodium sulfate and concentrated to dryness. The solid was chromatographed on a column of silica gel eluting with a 1:1 mixture of ethyl acetate:hexane containing 0.7% acetic acid to give 1.2 g (37% yield) of the title compound as a beige solid.

6-Acetylamino-2-oxindole (O-59)

6-Acetylamino-2-oxindole was synthesized using the procedure set forth in Helv. Chem. Acta 20:373, 1937.

6-(3-Trifluoroacetamidophenyl)-2-oxindole (O-60)

3-Aminophenylboronic acid (3.9 g), 5 g of 5-bromo-2-fluronitrobenzene, 0.8 g of tetrakis(triphenylphosphine)-palladium and 23 mL of 2 M sodium bicarbonate solution in 50 mL of toluene under nitrogen was refluxed for 2.5 hours. The reaction was poured into 200 mL of ice water and the mixture extracted three times with 50 mL of ethyl acetate each time. The combined organic layers were washed with 50 mL of water and 20 mL of brine, dried over anhydrous sodium sulfate and concentrated to give 9.7 g (92 % yield) of 2-fluoro-5-(3-aminophenyl)nitrobenzene as a dark brown oil.

Trifluoroacetic anhydride (5.4 mL) was slowly added to a stirred solution of 9.7 g, of 2-fluoro-5-(3-aminophenyl)-nitrobenzene and 5.3 mL of triethylamine in 50 mL of dichloromethane at 0 °C and the mixture was stirred for an additional 20 minutes. The mixture was concentrated and the residue chromatographed on a column of silica gel eluting with 10% ethyl acetate in hexanes to give 8.6 g (65% yield) of 2-fluoro-5-(3-trifluoroacetyamidophenyl)-nitrobenzene as a pale orange oil which solidified on standing.

Dimethyl malonate (9.6 mL) was added dropwise to a stirred suspension of 3.2 g of 60% sodium hydride in mineral oil in 40 mL of anhydrous dimethylsulfoxide under nitrogen. The mixture was stirred for 10 minutes and 2-fluoro-5-(3-trifluoroacetamidophenyl)nitrobenzene in 20 mL of dimethylsulfoxide was added. The resulting dark red mixture was heated to 100 °C for 2 hours. The reaction was quenced by pouring into 100 mL of saturated ammonium chloride solution and extracted twice with 50 mL of ethyl acetate each time. The organic phase was washed with 50 mL each of saturated ammonium chloride solution, water, and brine, dried over anhydrous sodium sulfate and concentrated to a yellow oil. The oil was chromatographed on a column of silica gel eluting with a 1:4 mixture of ethyl acetate:hexane to give 4.4 g (50% yield) of dimethyl 2-[2-nitro-4-(3-trifluoroacetamidophenyl)phenyl]malonate as a pale yellow solid.

Dimethyl 2-[2-nitro-4-(3-trifluoroacetamidophenyl)-phenyl]malonate (4.4 g) was refluxed overnight in 50 mL of 6 N hydrochloric acid. The reaction mixture was cooled to room temperature and the solids were collected by vacuum filtration, washed with water,

and dried under vacuum to give 2.7 g (73% yield) of 2-[2-nitro-4-(3-trifluoroacetamidophenyl)phenyl]acetic acid.

2-[2-Nitro-4-(3-trifluoroacetamidophenyl)phenyl]acetic acid (100 mg) and 50 mg of iron powder in 3 mL of acetic acid was heated to 100 °C for 2 hours. The reaction mixture was concentrated and the residue sonicated in 5 mL of ethyl acetate. The insoluble solids were removed by vacuum filtration and the filtrate washed with 1 N hydrochloric acid, water and brine, dried over anhydrous sodium sulfate and concentrated to give 10 mg (14% yield) of the title compound as a rose-colored solid.

B. Synthesis of Aldehydes

Synthesis of 6,7,8,9,-Tetrahydro-5*H*-carbazole-3-carbaldehyde:

1. The corresponding non-commercially available substituted tetrahydrocarbazoles were prepared using published procedures (*J. Chem. Soc.* **1945**, 530; *Aust. J. Chem.* **1952**, 976; and *J. Chem. Soc.* **1950**, *84*, 94).

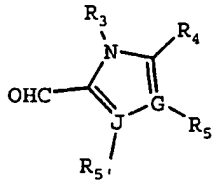
2. Vilsmeier formylation (Org. Synth. Coll., Vol. IV, 1963, 831) of the substituted 6,7,8,9,-Tetrahydro-5*H*-carbazoles from step 1 gives the final substituted tetrahydrocarbazole-3-carboxaldehydes.

Synthesis of Substituted 9*H*-Carbazole-3-carbaldehydes, Dibenzothiphen-2-carboxaldehydes, Dibenzofuran-2-carboxaldehydes, 6,7,8,9-Tetrahydro-dibenzofuran-2-carbaldehyde, 9*H*- -Carboline-6-carbaldehyde, 9*H*-2,4,9-Triaza-fluorene-6-carbaldehyde, 9*H*-Pyrido[2,3-b]indole-6-carbaldehyde, 9-Thia-1,5,7-triaza-fluorene-3-carbaldehyde, and Furo[3,2-*b*;4,5-*b* ']dipyridine-2-carbaldehyde:

Substituted 9*H*-carbazole-3-carbaldehydes, dibenzothiphen-2-carboxaldehydes, and dibenzofuran-2-carboxaldehydes are prepared by conventional Vilsmeier formylation starting from commercially available substituted 9H-carbazole, dibenzothiphen, dibenzofuran, 6,7,8,9-tetrahydro-dibenzofuran, 9*H*--ca r b oline, 9*H*-2,4,9-triaza-fluorene, 9*H*-Pyrido[2,3-b]indole-6-carbaldehyde, 9-Thia-1,5,7-triaza-fluorene-3-carbaldehyde, and Furo[3,2-*b*;4,5-*b* ']dipyridine.

EXAMPLE 4: PROCEDURES FOR SYNTHESIZING THE IMIDAZOYL INDOLINONE COMPOUNDS OF THE INVENTION

The compounds of this invention, as well as the precursor 2-indolinones and carbonylimidazoles, wherein a "carbonylimidazole" refers to a compound having the structure:



may be readily synthesized using techniques well known in the chemical arts. It will be appreciated by those skilled in the art that other synthetic pathways for forming the compounds of the invention are available and that the following is offered by way of example and not limitation.

A. General Synthetic Procedure

The following general methodology may be employed to prepare the compounds of this invention:

The appropriately substituted 2-indolinone (1 equiv.), the appropriately substituted carbonylimidazole (1.2 equiv.) and piperidine (0.1 equiv.) are mixed with ethanol (1-2 mL/mmol 2-indolinone) and the mixture is then heated at 90 °C for 3 to 5 hours. After cooling, the precipitate is filtered, washed with cold ethanol and dried to yield the target compound.

B. Specific Syntheses

The following examples show representative syntheses of several of the 2-indolinones of this invention. These 2-indolinones, as well as the others claimed, will form the claimed compounds by reaction with an appropriately substituted carbonylimidazole using the above procedure.

Preparation of 5-Bromo-2-indolinone

2-indolinone (1.3 g) in 20mL of acetonitrile was collected to -10 °C and 2.0 g of N-bromosuccinimide was slowly added with stirring. The reaction was stirred for 1 hour at 10 °C and 2 hours at 0 °C. The precipitate was collected, washed with water and dried to give 1.9 g (90% yield) of the title compound.

Preparation of 5-Nitro-2-indolinone

The 2-indolinone (6.5 g) was dissolved in 25 mL of concentrated sulfuric acid and the mixture maintained at -10-15 °C while 2.1 mL of fuming nitric acid was added dropwise. After the addition of the nitric acid the reaction mixture was stirred at 0 °C for 0.5 hr and poured into ice-water. The precipitate was collected by filtration, washed with water and crystallized from 50% of acetic acid. The final crystal was then filtered, washed with water and dried under vacuum to give 6.3 g (70%) of 5-nitro-2-indolinone.

Preparation of 5-Carboxyethyl-2-indolinone

Step 1: Synthesis of 5-Chloroacetyl-2-indolinone

Aluminum chloride (30.8g) and 2-oxindole (5.0 g) were added to 200 mL of carbon disulfide at room temperature and the mixture stirred. Choloroacetyl chloride (3.8 mL) was added and the stirring continued for 1 hour. The mixture was heated to reflux for 3 hours, cooled and the solvent decanted. The residue was stirred in ice water until it became a solid suspension. The solid was collected by vacuum filtration, washed with water, and dried to give 7.0 g (90% yield) of the title compound.

Step 2: Synthesis of 5-Chloroethyl-2-indolinone

(indolinone-041) 5-Chloroacetyl-2-indolinone (7.0 g) was added to 25 mL of trifluoroacetic acid and the mixture cooled in an ice bath with stirring. Triethylsilane (12.3 mL) was added drop-wise over 2 minutes. The reaction was then stirred at room temperature for 4 hours and poured into ice water. Hexane was added, the mixture stirred vigorously, and the solid collected by vacuum filtration and washed with hexane to give 5.9 g (91% yield) of the product as a white solid.

Step 3: Synthesis of 5-Cyanoethyl-2-indolinone

Potassium cyanide (2.02 g) was added to 15 mL of dimethylsulfoxide and heated to 90 °C. 5-Chloroethyl-2-indolinone (3.0 g) dissolved in 5 mL of dimethyl sulfoxide was added slowly with stirring, and the reaction heated to 150 °C for 2 hours. The mixture was cooled, poured into ice water and the precipitate collected by vacuum filtration, washed with water, and dried to give crude product. The crude material was chromatographed on silica gel in 5% methanol in chloroform to give 1.2 g (42% yield) of the title compound.

Step 4: Synthesis of 5-Carboxyethyl-2-indolinone

5-Cyanoethyl-2-indolinone (4.02 g) in 10 mL of water containing 25 mL of concentrated hydrochloric acid was refluxed for 4 hours. The mixture was cooled, water added and the resulting solid collected by vacuum filtration, washed with water and dried to give 1.9 g (44% yield) of the title compound as a yellow solid.

Preparation of 5-Aminosulfonyl-2-indolinone

Step 1: Synthesis of 5-chlorosulfonyl-2-indolinone

To the 100 mL of flask charged with 27 mL of chlorosulphonic acid was added 2-indolinone slowly. The reaction mixture was maintained between 25-30 °C. After addition of 2-indolinone, the reaction mixture was stirred at room temperature for 1.5 hr, then heated to 69 °C for 1 hr, cooled, and poured into ice water. The precipitate was filtered, washed with water and dried in a vacuum oven to give 11.0 g (50%) of 5-chlorosulfonyl-2-indolinone.

Step 2: Synthesis of 5-Aminosulfonyl-2-indolinone.

5-Cholorosulfonyl-2-indolinone (2.1 g) was added to 10 mL of ammonium hydroxide in 10 mL of ethanol and stirred at room temperature overnight. The mixture was concentrated and the solid collected by vacuum filtration to give 0.4 g (20% yield) of the title compound as an off-white solid.

Preparation of 5-(Morpholin-4-yl)sulfonyl)-2-indolinone

To a 100 mL flask charged with 27 mL of chlorosulfonic acid was added slowly 13.3 g of 2-indolinone. The reaction temperature was maintained below 30 °C during the

addition of oxindole. After addition of 2-indolinone, the reaction mixture was stirred at room temperature for 1.5 hr, heated to 68 °C for 1 hr, cooled, and poured into water. The precipitate was washed with water and dried in vacuum oven.

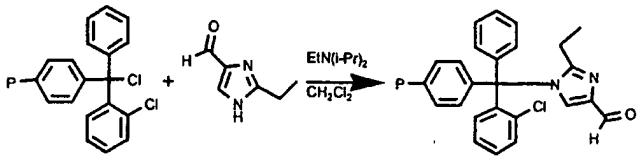
5-Chlorosulfonyl-2-indolinone (2.3 g) and 2.2 g morpholine in 50 mL of dichloromethane was stirred for 3 hours at room temperature. The precipitate was collected by vacuum filtration, washed with ethyl acetate and hexane and dried to give 2.09 g (74%) of the title compound as a white solid.

PREPARATION OF 3-(2-ETHYLIMIDAZOL-4-YL)-2-INDOLINONE BY IMMOBILIZED PHASE SYNTHESIS.

In addition to the above synthetic approach to the compounds of this invention, the following novel procedure wherein a carbonylimidazole is (1) reversibly bound to a solid substrate which is insoluble in the solvents used in the reaction and is of sufficient size to be manipulated by, without limitation, filtration and other physical procedures, (2) reacted with a 2-indolinone, (3) freed of residual starting materials and non-immobilized byproducts and (4) released from the solid substrate and isolated. The following is an example of this procedure which may be employed to prepare any of the compounds of this invention.

A. Attachment of 2-Ethyl-4-formylimidazole to 2-Chlorotritylchloride Resin

A mixture of 2-chlorotritylchloride resin (2, 100mg, 0.67 mmol/g loading capacity), N,N-diisopropylethylamine (87 mg, 0.67 mmol), and dichloromethane (1 mL) was gently stirred for 45 min. at ambient temperature. To the stirred mixture was then added 2-ethyl-4-formylimidazole (3, 25 mg, 0.201 mmol). After stirring for 21h, the resin was filtered and washed with 30 mL each of acetone, water, dichloromethane, methanol, and another portion of acetone. The resin was air dried for 15 min.



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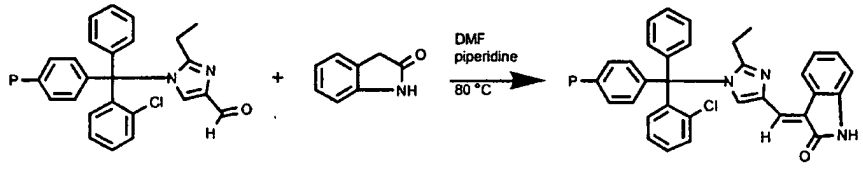
B. Synthesis of Resin-Attached Imidazolyl Indolinone

A mixture of 2-ethyl-4-formylimidazole attached to 2-chlorotrityl resin (4, 78 mg, 0.052 mmol based on 0.67 mmol/g loading), DMF (0.9 mL) and piperidine (0.1 mL) was gently stirred for 45 min. at ambient temperature, afterwhich was added 2-indolinone (5, 70 mg, 0.523 mmol). The resultant mixture was gently stirred and heated at 80 °C for 20h. The resin was filtered and washed with 30 mL each of acetone, water, dichloromethane, methanol, and another portion of acetone. Finally, the resin was air dried for 20 min.



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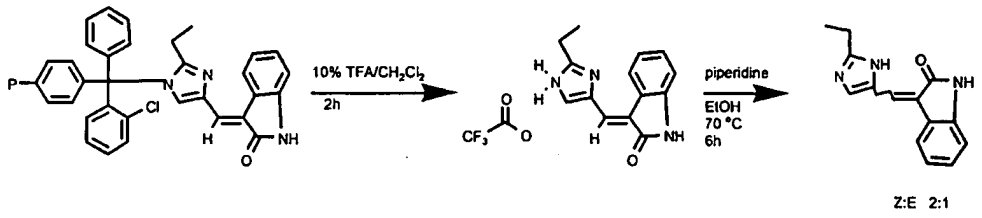
C. Cleavage of Resin-Attached Imidazolyl Indolinone

A mixture of resin-attached imidazolyl indolinone (6, 64 mg, 0.043 mmol based on 0.67 mmol/g loading), dichloromethane (0.9 mL), and trifluoroacetic acid (0.1 mL) was vortexed every 15 min for 2h at ambient temperature. The mixture was filtered, and the resin was washed with MeOH (1 mL). Concentration of the filtrate provided the TFA salt of the imidazolyl indolinone (7, 12 mg, 0.034 mmol, 79%) as a 10:1 E/Z isomeric mixture. Treatment of the mixture with piperidine (0.1 mL) and EtOH (0.9 mL) at 70 °C for 6h afforded a 2:1 Z/E isomeric mixture of imidazolyl indolinones (8).



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EXAMPLE 5: SYNTHESIS OF PHENYL-2-INDOLINONE COMPOUNDS

The compounds of this invention may be synthesized using the procedure described below. Other approaches to the synthesis of the compounds and/or precursors to the compounds of this invention may become apparent to those skilled in the art based on the disclosures herein. Such alternate procedures are within the scope and spirit of this invention. The exemplary syntheses which follow are not to be construed as limiting the scope of this invention in any manner.

Compound PI-001: 3-(3,5-Dimethyl-1H-pyrrol-2-ylmethylene)-6-(3-methoxyphenyl)-1,3-dihydroindol-2-one

Tetrakis(triphenylphosphine)palladium (0.8 g) was added to a mixture of 5 g of 3-methoxyphenylboronic acid, 5 g of 5-bromo-2-fluoronitrobenzene and 11 mL of 2 M sodium carbonate in 100 mL of toluene. The mixture was refluxed for 2 hours, diluted with water and extracted with ethyl acetate. The ethyl acetate layer was separated, washed with saturated sodium bicarbonate then with brine, dried and concentrated to give an oily solid. The solid was chromatographed on silica gel using ethyl acetate:hexane 1:6 as the eluent to give 4.3 g (77 % yield) of 4-fluoro-3'-methoxy-3-nitrobiphenyl.

Dimethyl malonate (9.7 mL) was added dropwise to 2.0 g of sodium hydride suspended in 50 mL of dimethylsulfoxide. The mixture was stirred at 100 °C for 35 minutes and then cooled to room temperature. 4-Fluoro-2'-methoxy-3-nitrobiphenyl (4.2 g) in 50 mL of dimethylsulfoxide was added and the mixture was again heated to 100 °C and stirred for 1 hour. The reaction mixture was cooled, quenched with 300 mL of

saturated ammonium chloride and extracted twice with ethyl acetate. The extracts were combined, washed with brine, dried over anhydrous sodium sulfate and concentrated to give crude dimethyl 3'-methoxy-3-nitrobiphenyl-4-malonate as a pale yellow solid.

The crude dimethyl 3'-methoxy-3-nitrobiphenyl-4-malonate was added to 45 mL of 6 N hydrochloric acid and the mixture was stirred at 110 °C for 4 days after which it was cooled to room temperature. The precipitate was collected by filtration, washed with water and hexane, and dried to give 5.3 g of 3'-methoxy-2-nitrobiphenyl-4-acetic acid as a light tan solid.

3'-Methoxy-3-nitrobiphenyl-4-acetic acid (5.2 g) was dissolved in methanol and hydrogenated at room temperature for 3 hours using 0.8 g of 10 % palladium on carbon as the hydrogenation catalyst. The catalyst was removed by filtration, washed with methanol and the filtrates combined and concentrated to give a brown solid. The solid was chromatographed on silica gel using ethyl acetate:hexane:acetic acid 33:66:1 as the eluent to give 3.0 g (75 % yield based on 4-fluoro-3'-methoxy-3-nitrobiphenyl) of 6-(3-methoxyphenyl)-2-oxindole as a pink solid.

^{1}HNMR (360 MHz, DMSO-d6) δ: 10.39 (s, br, 1H, NH), 7.35 (t, J = 7.85Hz, 1H), 7.26 (d, J = 7.78Hz, 1H), 7.19 (dd, J = 1.22, 7.8Hz, 1H), 7.13-7.16 (m,1H), 7.09-7.1 (m,1H), 7.01 (d, J = 1.48Hz, 1H), 6.90-6.93 (m, 1H), 3.8 (s, 3H, OCH_3), 3.49 (s, 2H, CH_2). MS m/z (relative intensity, %): 240.0 (100, $[M+1]^+$).

3,5-Dimethyl-1H-pyrrole-2-carboxaldehyde (74 mg), 120 mg 6-(3-methoxyphenyl)-2-oxindole and 1 drop piperidine in 2 mL ethanol were heated to 95 °C and held overnight. The reaction mixture was cooled and the precipitate that formed was filtered, washed with cold ethanol and hexane and dried in a vacuum oven overnight to give 130 mg of the title compound (75%) as a brown solid.

^{1}HNMR (360 MHz, DMSO-d6) δ: 13.38 (s, br, 1H, NH-1'), 10.82 (s, br, 1H, NH-1), 7.77 (d, J = 7.88Hz, 1H, H-4), 7.58 (s, 1H, H-vinyl), 7.35 (t, J = 7.94Hz, 1H), 7.27 (dd, J = 1.58, 8.12Hz, 1H), 7.19 (d, J = 7.72Hz, 1H), 7.13-7.15 (m, 1H), 7.09 (d, J = 1.21Hz, 1H), 6.9 (dd, J = 2.4, 7.91Hz, 1H), 6.01 (d, J = 1.86Hz, 1H, H- 4'), 3.82 (s, 3H, OCH_3), 2.32 (s, 3H, CH_3), 2.31 (s, 3H, CH_3). MS m/z (relative intensity, %): 345.0 (100, $[M+1]^+$).

Compound PI-002: 6-(3-Methoxyphenyl)-3-(4,5,6,7-tetrahydro-1H-indol-2-ylmethylene)-1,3-dihydroindol-2-one

To a stirred suspension of 2.3 g of 1:1 dimethylformamide/ phosphorus oxychloride (Vilsmeier reagent) in 20 mL of dichloromethane at 0 °C was added 2 g of 4,5,6,7-tetrahydroindole in portions, keeping the temperature below 10 °C. The clear mixture was refluxed for 45 minutes and then cooled to 4 °C. Water (30 mL) was added and the aqueous layer separated and saved. The organic layer was extracted with 10 mL of water and the aqueous extracts were combined and cooled to 4 °C. 25 mL of 2.5 N sodium hydroxide was slowly added to the stirred aqueous layer and the mixture was then stirred for one hour during which time the temperature was maintained at about 4 °C. The light tan precipitate which formed was collected by vacuum filtration and washed with water until the pH of the wash was between 7 and 8. The product was sucked dry on the funnel and then vacuum dried to give 1 g of 4,5,6,7-tetrahydro-1H-indole-2-carboxaldehyde (41%) as a tan solid.

^{1}HNMR (360 MHz, DMSO-d6) δ: 11.55 (s, br, 1H, NH), 9.25 (s, 1H, CHO), 6.65 (d, J = 2.27 Hz, 1H, H-3), 2.55 (t, J = 5.93Hz, 2H), 2.44 (t, J = 5.93HZ, 2H), 1.64-1.73 (m, 4H, H-5,6). MS (FAB) m/z (relative intensity, %) 150.2 (100, $[M+1]^+$).

4,5,6,7-Tetrahydro-1H-indole-2-carboxaldehyde (90 mg), 120 mg of 6-(3-methoxyphenyl)-2-oxindole and 1 drop of piperidine in 2 mL of ethanol were held at 95 °C overnight. The reaction mixture was cooled and the precipitate which formed was filtered, washed with cold ethanol and hexane and dried in a vacuum oven overnight to give 170 mg of the title compound (92%) as a brown solid.

^{1}HNMR (360 MHz, DMSO-d6) δ: 13.1 (s, br, 1H, NH-1'), 10.83 (s, br, 1H, NH-1), 7.64 (d, J = 8.33Hz, 1H, H-4), 7.62 (s, 1H, H-vinyl), 7.35 (t, J = 7.95Hz, 1H), 7.28 (dd, J = 1.47, 7.68Hz, 1H), 7.19 (d, J = 7.54Hz, 1H), 7.13-7.14 (m, 1H), 7.09 (d, J = 1.8Hz, 1H, H-7), 6.9 (dd, J = 2.6Hz, 7.65Hz, 1H), 6.6 (s, br, 1H, H- 3'), 3.82 (s, 3H, OCH_3), 2.71 (t, J = 6.04Hz, 2H), 2.54 (t, J = 6.04Hz, 2H), 1.7 - 1.79 (m, 4H). MS m/z (relative intensity, %): 371.4 (100, $[M+1]^+$).

Compound PI-003: 3-(3,5-Diethyl-1H-pyrrol-2-ylmethylene)-6-(3-methoxyphenyl)-1,3-dihydroindol-2-one

Dimethylformamide (50.2 g) and 200 mL of dichloromethane were charged to a 2 l flask fitted with a stirrer, an addition funnel, and a thermocouple and cooled to -4 °C. Phosphorus oxychloride (105.6 g) in 450 mL methylene chloride was added over a few minutes and the reaction stirred for 45 minutes at room temperature. The mixture was then cooled to -4 °C and 76.9 g 2,4-diethylpyrrole in 150 mL dichloromethane was added dropwise with stirring over 13 minutes. At the end of the addition, the temperature was 14 °C. The reaction was stirred for 9.5 hours during which time it was allowed to come to room temperature. Thin layer chromatography showed no starting material. The reaction mixture was heated to reflux for 10 minutes and then cooled to room temperature. Sodium hydroxide (40 g in 700 mL of water) and acetic acid (120 g) were added over 5 minutes with stirring. When spontaneous reflux subsided, the reaction was refluxed for 30 minutes, 40 g of solid sodium acetate was added and reflux was continued for 15 additional minutes. Gas chromatography showed only a single product to be present. The green organic layer was separated and washed 3 times with 75 mL saturated sodium carbonate during which the solution turned brown. The solution was dried over anhydrous sodium sulfate, rotary evaporated, and distilled at 0.6 - 1.0 mm through a 10 inch vacuum jacketed Vigreux column to give 96.4g (94% yield) of 2,4-diethyl-5-formylpyrrole, BP 108 - 113 °C. The product was light yellow but darkened rapidly in the collection flask.

^{1}HNMR (360 MHz, DMSO-d6) δ: 11.45 (s, br, 1H, NH), 9.46 (s, 1H, CHO), 5.9 (d, J = 2.32Hz, 1H, H-4), 2.67 (q, J = 7.55Hz, 2H), 2.54 (q, J = 7.55Hz, 2H), 1.15 (t, J = 7.55HZ, 6H). APCI MS m/z (relative intensity, %): 151.8 (100, M^+).

3,5-Diethyl-1H-pyrrole-2-carboxaldehyde (90 mg), 120 mg 6-(3-methoxyphenyl)-2-oxindole and 1 drop piperidine in 2 mL ethanol were heated to 95 °C and held at that temperature overnight. The reaction mixture was cooled and the precipitate which formed was filtered, washed with cold ethanol and hexane and dried in a vacuum oven overnight to give 162 mg of the 3-(3,5-Diethyl-1H-pyrrol-2-ylmethylene)-6-(3-methoxyphenyl)-1,3-dihydroindol-2-one (87%) as a brown solid.

^{1}HNMR (360 MHz, DMSO-d6) δ: 13.52 (s, br, 1H, NH-1'), 10.83 (s, br, 1H, NH-1), 7.78 (d, J = 8.27Hz, 1H, H-4), 7.6 (s, 1H, H-vinyl), 7.35 (t, J = 8.45Hz, 1H), 7.27 (dd, J = 1.67, 7.97Hz, 1H), 7.19 (d, J = 7.73Hz, 1H), 7.13-7.15 (m, 1H), 7.1 (d, J = 1.29Hz, 1H, H-7), 6.9 (dd, J = 2.16, 8.17Hz, 1H), 6.1 (d, J = 2.54Hz, 1H, H- 4'), 3.82 (s, 3H, OCH_3), 2.65 - 2.8 (m, 4H, CH_3CH_2), 1.17 - 1.28 (m, 6H, CH_3CH_2). MS m/z (relative intensity, %): 373.3 (100, $[M+1]^+$).

Compound PI-004: 3-(3H-Imidazol-4-ylmethylene)-6-(3-methoxyphenyl)-1,3-dihydroindol-2-one

3H-Imidazole-4-carboxaldehyde (108.6 mg), 120 mg of 6-(3-methoxyphenyl)-2-oxindole and 1 drop of piperidine in 2 mL of ethanol were held at 95 °C overnight. The reaction mixture was cooled and the precipitate which formed was filtered, washed with cold ethanol and hexane and dried in a vacuum oven overnight to give 140 mg of the title compound (88%) as a brown solid.

^{1}HNMR (360 MHz, DMSO-d6) δ: 13.68 (s, br, 1H, NH-1'), 11.04 (s, br, 1H, NH-1), 8.01 (s, br, 1H), 7.86 (s, br, 1H), 7,73 (d, J = 8.07Hz, 1H), 7.65 (s, br, 1H), 7.31-7.39 (m, 2H), 7.11-7.21 (m, 3H), 6.92-6.94 (m, 1H), 3.82 (s, 3H, OCH_3). MS m/z (relative intensity, %) 318.1 (100, $[M+1]^+$).

Compound PI-005: 4-{4-[6-(3-Methoxyphenyl)-2-oxo-1,2-dihydroindol-3-ylidenemethyl]-phenyl}-piperazine-1-carboxaldehyde

To a solution of 30 mL dimethylformamide in 200 mL dichloroethane cooled to 0 °C was added 30 mL phosphorus oxychloride. The mixture was stirred for 30 minutes and 16 g of 1-phenylpiperazine was added slowly over 15 minutes. The mixture was stirred at 50 °C for 1 hour. Thin layer chromatography (ethyl acetate:acetone:hexane, 2:1:1, silica gel) showed no starting material but a major product (Rf 0.4) and a minor product (Rf 0.5). The reaction mixture was poured into ice water, adjusted to pH 9 with aqueous sodium hydroxide and stirred for 1 hour. The mixture was extracted with ethyl acetate and the extract washed with brine, dried over anhydrous sodium sulfate and concentrated. The

residue was chromatographed on a silica gel column eluting with ethyl acetate:acetone:hexane (2:1:1) to give 9.0 g (41 % yield) of 4-(4-formylphenyl)-piperazine-1-carboxaldehyde.

4-(4-Formylphenyl)piperazine-1-carboxaldehyde (131 mg), 120 mg 6-(3-methoxyphenyl)-2-oxindole and 1 drop of piperidine in 2 mL ethanol were held at 95 °C overnight. The reaction mixture was cooled and the precipitate which formed was filtered, washed with cold ethanol and hexane and dried in a vacuum oven overnight to give 200 mg of the title compound (91%) as a brown solid.

^{1}HNMR (360 MHz, DMSO-d6) δ: 10.54 (s, br, 1H, NH-1), 8.44 (d, J = 8.79Hz, 2H), 8.1 (s, 1H), 7.69-7.71 (2H), 7.361 (t, J = 8.05Hz, 1H), 7.26 (dd, J = 1.55, 7.95Hz, 1H), 7.19-7.2 (m, 1H), 7.14-7.15 (m, 1H), 7.02-7.05 (m, 3H), 6.9-6.93 (m, 1H), 3.82 (s, 3H, OCH_3), 2.35 - 3.53 (m, 8H, H- piperazine). MS m/z (relative intensity, %): 440.0 (100, $[M+1]^+$).

Compound PI-006: 6-(3-Methoxyphenyl)-3-(3-methyl-4,5,6,7-tetrahydro-1H-indol-2-ylmethylene)-1,3-dihydroindol-2-one

Acetic acid (34 mL), 18 g 2-acetylcyclohexanone, 28.5 g diethyl aminomalonate hydrochloride and 11.6 g sodium acetate were charged to a 250 mL three neck flask equipped with a reflux condenser and magnetic stirring. The flask was placed in an oil bath and the bath temperature was raised to 130 °C over 20 minutes. At 80 °C carbon dioxide began to evolve. Fifteen minutes after reaching 130 °C, gas evolution ceased. The mixture was cooled in an ice bath and 68 mL water was slowly added with stirring. The tan precipitate which formed was collected by vacuum filtration and washed twice with water and twice with cold ethanol:water 2:1 to give 20.8 g (78 % yield) of 2-ethoxycarbonyl-3-methyl-4,5,6,7-tetrahydroindole as an off-white solid. Thin layer chromatography (dichloromethane, silica gel) revealed two spots, Rf 0.6 and 0.5. Proton NMR revealed that the spots were the desired product (Rf 0.6) and its regioisomer (Rf 0.5) in a ratio of 7:1. Recrystallization from hexane (about 8 mL/g) gave 12.6 g (70 % yield) of 2-ethoxycarbonyl-3-methyl-4,5,6,7-tetrahydroindole as an off-white solid. NMR of the recrystallized produce showed that the ratio of the desired product to its regioisomer had

increased to 43:1.

2-Ethoxycarbonyl-3-methyl-4,5,6,7-tetrahydroindole (15.3 g), 104 mL 2.5 N sodium hydroxide, 125 mL ethanol and 180 mL water were charged to a 500 mL round bottom flask equipped with a reflux condenser and magnetic stirring. The flask was placed in an oil bath and heated to reflux. The mixture was refluxed for 5 hours at which time a small amount of dark oil formed. The mixture was diluted with 100 mL of water and cooled to 2 °C. About 0.5 g of the solidified oil was removed by filtration. The oil contained starting material and 3-methyl-4,5,6,7-tetrahydro- indole. Fifty mL of 6 N hydrochloric acid were added with stirring. The off-white precipitate which formed and which quickly turned pink was collected by vacuum filtration, washed with water and vacuum dried at ambient temperature for 3 days to give 14.5 g (111 % yield) of 2-carboxy-3-methyl-4,5,6,7-tetrahydroindole.

2-Carboxy-3-methyl-4, 5, 6, 7-tetrahydroindole (14.5 g) was placed in a 50 mL round bottom flask equipped with magnetic stirring. The flask was placed in an oil bath and heated to a bath temperature of 140–150 °C for 20 minutes. Water was seen to condense at the neck of the flask. Thin layer chromatography (dichloromethane, silica gel, Rf 0.9) showed complete conversion to product. The dark liquid was cooled, taken up in 30 mL of hexane, dried over sodium sulfate and evaporated to give 7.5 g of 3-methyl-4,5,6,7-tetrahydroindole as a mixture of crystals and dark oil.

To a stirred suspension of 5.0 g of Vilsmeier reagent in 40 mL dichloromethane at 0 °C was added 4.8 g of 3-methyl-4,5,6,7-tetrahydroindole in portions so as to keep the temperature below 10 °C. The clear mixture was refluxed for 45 minutes and then cooled to 4 °C. Water (80 mL) was added and the aqueous layer separated and saved. The organic layer was extracted with 20 mL of water and the aqueous extracts combined and cooled to 4 °C. Sixty-two mL of 2.5 N sodium hydroxide was slowly added to the stirred aqueous layer after which the mixture allowed to stir for one hour at 4 °C. The light tan precipitate which formed was collected by vacuum filtration and washed with water until the pH of the wash was between 7 and 8. The product was sucked dry on filter funnel and then vacuum dried to give 4.3 g of 2-formyl-3-methyl-4, 5, 6, 7-tetrahydroindole as a tan solid. Thin layer chromatography (ethyl acetate, silica gel), Rf 0.75. Some additional product (0.7 g) of similar purity was collected directly from the mother liquor without concentrating. It was vacuum dried to give a total of 5.0 g (86 % yield) of 3-Methyl-

4,5,6,7-tetrahydro-1H-indole-2-carboxaldehyde.

^{1}HNMR (360 MHz, DMSO-d6) δ: 11.25 (s, br, 1H, NH), 9.43 (s, 1H, CHO), 2.49-2.51 (m, 2H), 2.23-2.33 (m, 2H), 2.14 (s, 3H, CH_3), 1.67-1.68 (m, 4H). MS m/z (relative intensity, %): 164 (100, $[M+1]^+$).

3-Methyl-4, 5, 6, 7-tetrahydro-1H-indole-2-carboxaldehyde (98 mg), 120 mg 6-(3-methoxyphenyl)-2-oxindole and 1 drop piperidine in 2 mL of ethanol were held at 95 °C overnight. The reaction mixture was cooled and the precipitate which formed was filtered, washed with cold ethanol and hexane and dried in a vacuum oven overnight to give 166 mg of the title compound (86%) as a brown solid.

^{1}HNMR (360 MHz, DMSO-d6) δ: 13.25 (s, br, 1H, NH-1'), 10.79 (s, br, 1H, NH-1), 7.77 (d, J = 8.03Hz, 1H, H-4), 7.6 (s, 1H, H-vinyl), 7.35 (t, J = 7.96Hz, 1H), 7.27 (dd, J = 1.66, 8.4Hz, 1H), 7.19 (d, J = 8Hz, 1H), 7.13-7.14 (m, 1H), 7.08 (d, J = 1.35Hz, 1H, H-7), 6.9 (dd, J = 2.27, 8.08Hz, 1H), 3.82 (s, 3H, OCH_3), 2.68 (t, J = 5.66Hz, 2H), 2.42 (t, J = 5.66Hz, 2H), 2.22 (s, 3H, CH_3), 1.7 - 1.79 (m, 4H). MS m/z (relative intensity, %): 385.1 (100, $[M+1]^+$).

Compound PI-007: 3-(3,5-Dimethyl-1H-pyrrol-2-ylmethylene)-6-phenyl-1,3-dihydroindol-2-one

Dimethyl malonate (10 mL) in 25 mL dimethylsulfoxide was added dropwise to 3.5 g sodium hydride suspended in 25 mL dimethylsulfoxide and the mixture was stirred at 100 °C for 10 minutes. The mixture was then cooled to room temperature and 4.7 g 4-fluoro-3-nitrobiphenyl in 25 mL dimethylsulfoxide was added. The mixture was again heated to 100 °C and then stirred for 2 hours, cooled and quenched with 300 mL saturated ammonium chloride solution. The mixture was extracted three times with ethyl acetate, the combined organic layers were washed with water and brine and evaporated to give crude dimethyl-3-nitrobiphenyl-4-malonate as a yellow oil.

The crude dimethyl-3-nitrobiphenyl-4-malonate was refluxed in 30 mL 6 N hydrochloric acid for 24 hours. The precipitate which formed was collected by vacuum filtration, washed with water and dried to give 4.5 g (80 % based on 4-fluoro-3-nitrobiphenyl) of 3-nitrobiphenyl-4-acetic acid as a cream colored solid.

Iron powder (2.6 g) was added all at once to 4.5 g 3-nitrobiphenyl-4-acetic acid in 40 mL acetic acid. The mixture was refluxed for 2 hours, concentrated to dryness and then taken up in ethyl acetate. The solids were removed by filtration and the filtrate was washed twice with 1 N hydrochloric acid followed by brine. The ethyl acetate solution was then dried over anhydrous sodium sulfate. The sodium sulfate was removed by filtration and the filtrate was concentrated to give 3.4 g (93% yield) 6-phenyl-2-oxindole as a light brown solid.

^{1}H NMR (360 MHz, DMSO-d6) δ: 10.4 (s, br, 1H, NH), 7.57-7.6 (m, 2H), 7.42-7.46 (m, 2H), 7.32-7.37 (m, 1H), 7.27 (d, J = 7.7Hz, 1H, H-4), 7.19 (dd, J = 1.6, 7.7Hz, 1H, H-5), 7.01 (d, J = 1.6Hz, 1H, H-7), 3.49 (s, 2H, CH_2). MS m/z (relative intensity, %): 210 (100, $[M+1]^+$).

3,5-Dimethyl-1H-pyrrole-2-carboxaldehyde (74 mg), 105 mg 6-phenyl-2-oxindole and 1 drop piperidine in 2 mL ethanol were held at 90 °C overnight. The reaction mixture was cooled and the precipitate which formed was filtered, washed with cold ethanol and hexane and dried in a vacuum oven overnight to give 100 mg of the title compound (64%) as an orange solid.

^{1}HNMR (360 MHz, DMSO-d6) δ: 13.33 (s, br, 1H, NH-1'), 10.84 (s, br, 1H, NH-1), 7.78 (d, J = 7.61Hz, 1H, H-4), 7.63 (d, J = 7.45Hz, 2H), 7.58 (s, 1H, H-vinyl), 7.44 (t, J = 7.58Hz, 2H), 7.31-7.35 (m, 1H), 7.27 (dd, J = 1.79, 7.7Hz, 1H), 7.1 (d, J = 1.41Hz, 1H, H-7), 6.01 (d, J = 2.36Hz, 1H, H- 4'), 2.32 (s, 3H, CH_3), 2.31 (s, 3H, CH_3). MS m/z (relative intensity, %): 314.2 (100, $[M+1]^+$).

Compound PI-008: 3-(3,5-Dimethyl-1H-pyrrol-2-ylmethylene)-6-(3-ethoxyphenyl)-1,3-dihydroindol-2-one

Tetrakis(triphenylphosphine)palladium (0.8 g) was added to a mixture of 4.2 g 3-ethoxyphenylboronic acid, 5.0 g 5-bromo-2-fluoronitrobenzene and 22 mL 2 M sodium carbonate in 50 mL toluene and 50 mL ethanol. The mixture was refluxed for 2 hours, concentrated, water was added and the mixture was extracted twice with ethyl acetate. The ethyl acetate layer was washed with water, then with brine and then dried and concentrated. The residue was chromatographed on silica gel using 5 % ethyl acetate in

hexane as the eluent to give 5.3 g (90 % yield) of crude 4-fluoro-3'-ethoxy-3-nitrobiphenyl as a yellow oil.

Dimethyl malonate (11.4 mL) was added dropwise to 4.0 g sodium hydride suspended in 20 mL dimethylsulfoxide. The mixture was stirred at 100 °C for 10 minutes and then cooled to room temperature. Crude 4-fluoro-3'-ethoxy-3-nitrobiphenyl (5.3 g) in 25 mL dimethylsulfoxide was added and the mixture was again heated to 100 °C and stirred for 2 more hours. The reaction mixture was then cooled and quenched with 300 mL of saturated ammonium chloride and extracted three times with ethyl acetate. The extracts were combined, washed with water and brine, dried over anhydrous sodium sulfate and concentrated to give crude dimethyl 3'-ethoxy-3-nitrobiphenyl-4-malonate as a yellow oil.

The crude dimethyl 3'-ethoxy-3-nitrobiphenyl-4-malonate was added to 60 mL 6 N hydrochloric acid, the mixture was heated to 100 °C and stirred at that temperature for 4 days. The reaction mixture was then cooled to room temperature. The precipitate which formed was collected by filtration, washed with water and hexane, and dried to give 4.7 g (77 % yield based on 5-bromo-2-fluoronitrobenzene) of crude 3'-ethoxy-3-nitrobiphenyl-4-acetic acid as a light tan solid.

Iron powder (2.4 g) was added in one portion to 4.6 g 3'-ethoxy-3-nitrobiphenyl-4-acetic acid in 40 mL glacial acetic acid and the mixture was refluxed for 2 hours. The reaction mixture was then concentrated to dryness, treated repeatedly with ethyl acetate and filtered to remove insoluble materials. The filtrate was washed twice with 1 N hydrochloric acid then with brine. It was then dried over anhydrous sodium sulfate and concentrated to give 3.5 g (91 % yield) of 6-(3-ethoxyphenyl)-2-oxindole as a light brown solid.

^{1}HNMR (360 MHz, DMSO-d6) δ: 10.4 (s, br, 1H, NH), 7.33 (t, J = 8.4Hz, 1H, H-3'), 7.35 (d, J = 7.77Hz, 1H), 7.19 (dd, J = 1.3, 7.66Hz, 1H), 7.13 (d, J = 7.69Hz, 1H), 7.07-7.08 (m, 1H), 7.0 (s, br, 1H), 6.9 (dd, J = 2.82, 8.08Hz, 1H), 4.08 (q, J = 7Hz, 2H, OCH_2CH_3), 3.49 (s, 2H, CH_2), 1.34 (t, J = 7Hz, 3H, OCH_2CH_3). MS m/z (relative intensity, %): 254.2 (100, $[M+1]^+$).

3,5-Dimethyl-1H-pyrrole-2-carboxaldehyde (74 mg), 105 mg 6-(3-ethoxyphenyl)-2-oxindole and 1 drop piperidine in 2 mL ethanol were held at 90 °C overnight. The

reaction mixture was cooled and the precipitate which formed was filtered, washed with cold ethanol and hexane and dried in a vacuum oven overnight to give 150 mg of the title compound (84%) as an orange solid.

^{1}HNMR (360 MHz, DMSO-d6) δ: 13.33 (s, br, 1H, NH-1'), 10.82 (s, br, 1H, NH-1), 7.77 (d, J = 8.28Hz, 1H, H-4), 7.58 (s, 1H, H-vinyl), 7.34 (t, J = 8.08Hz, 1H), 7.27 (dd, J = 1.76, 7.84Hz, 1H), 7.17 (d, J = 7.56Hz, 1H), 7.11-7.12 (m, 1H), 7.08 (d, J = 1.63Hz, 1H), 6.87-6.9 (m, 1H), 6.01 (d, J = 2.21Hz, 1H, H- 4'), 4.09 (q, J = 7Hz, 2H, CH_2CH_3), 2.32 (s, 3H, CH_3), 2.31 (s, 3H, CH_3), 1.35 (t, J = 7Hz, 3H, CH_2CH_3). MS m/z (relative intensity, %): 359.2 (100, $[M+1]^+$).

Compound PI-009: 6-(3-Ethoxyphenyl)-3-(4,5,6,7-tetrahydro-1H-indol-2-ylmethylene)-1,3-dihydroindol-2-one

4,5,6,7-Tetrahydro-1H-indole-2-carboxaldehyde (90 mg), 127 mg 6-(3-ethoxyphenyl)-2-oxindole and 1 drop piperidine in 2 mL ethanol were held at 90 °C overnight. The reaction mixture was cooled and the precipitate which formed was filtered, washed with cold ethanol and hexane and dried in a vacuum oven overnight to give 157 mg of the title compound (82%) as an orange solid.

^{1}HNMR (360 MHz, DMSO-d6) δ: 13.1 (s, br, 1H, NH-1'), 10.84 (s, br, 1H, NH-1), 7.63 (d, J = 9.06Hz, 1H, H-4), 7.62 (s, 1H, H-vinyl), 7.34(t, J = 7.9Hz,1H), 7.27 (dd, J = 1.66, 7.91Hz, 1H), 7.17 (d, J = 7.72Hz,1H), 7.1-7.12 (m, 1H), 7.08 (d, J = 1.1Hz, 1H), 6.89 (dd, J = 1.82, 8.08Hz, 1H), 6.6 (s, br, 1H, H- 3'), 4.09 (q, J = 7Hz, 2H, OCH_2CH_3), 2.71 (t, J = 6Hz, 2H), 2.52 (t, J = 6Hz, 2H), 1.7 - 1.79 (m, 4H), 1.34 (t, J = 7 Hz, 3H, OCH_2CH_3). MS m/z (relative intensity, %): 385.3 (100, $[M+1]^+$).

Compound PI-010: 6-(3-Ethoxyphenyl)-3-(3H-imidazol-4-ylmethylene)-1,3-dihydroindol-2-one

3H-Imidazole-4-carboxaldehyde (58 mg), 127 mg of 6-(3-ethoxyphenyl)-2-oxindole and 1 drop piperidine in 2 mL ethanol were heated to 95 °C and held overnight. The reaction mixture was cooled and the precipitate which formed was filtered, washed

with cold ethanol and hexane and dried in a vacuum oven overnight to give 130 mg of the title compound (78%) as a gold solid.

^{1}HNMR (360 MHz, DMSO-d6) δ: 13.69 (s, br, 1H, NH-1'), 11.06 (s, br, 1H, NH-1), 8.02 9s, br, 1H), 7.87 (s, br, 1H), 7.73 (d, J = 8.09Hz, 1H), 7.64 (s, br, 1H), 7.31-7.37 (m, 2H), 7.18 (d, J = 7.72Hz, 1H), 7.12 (d, J = 12.13Hz, 2H), 6.9-6.93 (m, 1H), 4.09 (q, J = 6.98 Hz, 2H, OCH_2CH_3), 1.34 (t, J = 6.98 Hz, 3H, OCH_2CH_3). MS m/z (relative intensity, %): 332.1 (100, $[M+1]^+$).

Compound PI-011: 6-(3-Ethoxyphenyl)-3-(5-methylthiophen-2-ylmethylene)-1,3-dihydroindol-2-one

5-Methylthiophene-2-carboxaldehyde (75.6 mg), 127 mg 6-(3-ethoxyphenyl)-2-oxindole and 1 drop piperidine in 2 mL of ethanol were heated to 95 °C and held overnight. The reaction mixture was cooled and the precipitate which formed was filtered, washed with cold ethanol and hexane and dried in a vacuum oven overnight to give 98 mg of the title compound (54%) as a brown solid.

^{1}HNMR (360 MHz, DMSO-d6) δ: 10.61 (s, br, 1H, NH-1), 8.01 (s, 1H, H-vinyl), 7.7-7.74 (m, 2H), 7.34 (t, J = 8.47Hz, 1H), 7.28 (dd, J = 1.49,7.97Hz, 1H), 7.18 (d, J = 7.76Hz, 1H), 7.13-7.7.14 (m, 1H), 7.04 (d, J = 1Hz, 1H), 6.89-6.95 (m, 2H), 4.09 (q, J = 6.98 Hz, 2H, OCH_2CH_3), 2.53 (s, 3H, CH_3), 1.34 (t, J = 6.98 Hz, 3H, OCH_2CH_3). MS m/z (relative intensity, %): 362.2 (100, $[M+1]^+$).

Compound PI-012: 3-(3,5-Diethyl-1H-pyrrol-2-ylmethylene)-6-(3-ethoxyphenyl)-1,3-dihydroindol-2-one

3,5-Diethyl-1H-pyrrole-2-carboxaldehyde (90.6 mg), 127 mg 6-(3-ethoxyphenyl)-2-oxindole and 1 drop piperidine in 2 mL ethanol were heated to 90 °C and held overnight. The reaction mixture was cooled and the precipitate which formed was filtered, washed with cold ethanol and hexane and dried in a vacuum oven overnight to give 149 mg of the title compound (77%) as an orange solid.

^{1}HNMR (360 MHz, DMSO-d6) δ: 13.52 (s, br, 1H, NH-1'), 10.82 (s, br, 1H, NH-1), 7.78 (d, J = 8.09Hz, 1H, H-4), 7.6 (s, 1H, H-vinyl), 7.34 (t, J = 8.09Hz, 1H), 7.27 (dd, J = 1.66, 7.91Hz, 1H), 7.18 (d, J = 7.73Hz, 1H), 7.12-7.13 (m, 1H), 7.0 (d, J = 1.47Hz, 1H), 6.87-6.9 (m, 1H), 6.1 (d, J = 2.57Hz, 1H, H- 4'), 4.09 (q, J = 6.98 Hz, 2H, OCH_2CH_3), 2.65 - 2.8 (m, 4H, CH_2 CH_3), 1.35 (t, J = 6.98 Hz, 3H, OCH_2CH_3), 1.18 - 1.27 (m, 6H, CH_2CH_3). MS m/z (relative intensity, %): 387.4 (100, $[M+1]^+$).

Compound PI-013: 6-Phenyl-3-(4,5,6,7-tetrahydro-1H-indol-2-ylmethylene)-1,3-dihydroindol-2-one

4,5,6,7-Tetrahydro-1H-indole-2-carboxaldhyde (90 mg), 105 mg 6-phenyl-2-oxindole and 1 drop piperidine in 2 mL ethanol were heated to 95 °C and held overnight. The reaction mixture was then cooled and the precipitate which formed was filtered, washed with cold ethanol and hexane and dried in a vacuum oven overnight to give 120 mg (70%) of the title compound as an orange solid.

^{1}HNMR (360 MHz, DMSO-d6) δ: 13.1 (s, br, 1H, NH-1'), 10.85 (s, br, 1H, NH-1), 7.61 (s, 1H, H-vinyl), 7.61-7.66 (m, 3H), 7.44 (t, J = 7.83Hz, 2H), 7.31-7.35 (m, 1H), 7.27 (dd, J = 1.58, 8.06Hz, 1H), 7.09 (d, J = 1.64Hz, 1H), 6.6 (s, br, 1H, H- 3'), 2.71 (t, J = 6.03Hz, 2H), 2.52 (t, J = 6.03Hz, 2H), 1.7 - 1.79 (m, 4H). MS m/z (relative intensity, %): 341.0 (50, $[M+1]^+$).

Compound PI-014: 3-(3H-Imidazol-4-ylmethylene)-6-phenyl-1,3-dihydroindol-2-one

3H-Imidazole-4-carboxaldehyde (58 mg), 105 mg 6-phenyl-2-oxindole and 1 drop piperidine in 2 mL ethanol were heated to 95 °C and held overnight. The reaction mixture was cooled and the precipitate which formed was filtered, washed with cold ethanol and hexane and dried in a vacuum oven overnight to give 78 mg of the title compound (54%) as a gold solid.

^{1}HNMR (360 MHz, DMSO-d6) δ: 13.69 (s, br, 1H, NH-1'), 11.08(s, br, 1H, NH-1), 8.02 (s, br, 1H), 7.87 (s, br, 1H), 7.74 (d, J = 8.05Hz, 1H), 7.63-7.65 (m, 3H), 7.46 (t, J

= 7.45Hz, 2H), 7.31-7.38 (m, 2H), 7.11 (s, br, 1H). MS m/z (relative intensity, %): 288.1 (81, $[M+1]^+$).

Compound PI-015: 3-(5-Methylthiophen-2-ylmethylene)-6-phenyl-1,3-dihydroindol-2-one

5-Methylthiophene-2-carboxaldehyde (75.6 mg), 105 mg 6-phenyl-2-oxindole and 1 drop piperidine in 2 mL ethanol were heated to 95 °C and held overnight. The reaction mixture was cooled and the precipitate which formed was filtered, washed with cold ethanol and hexane and dried in a vacuum oven overnight to give 93 mg of the title compound (59%) as an orange solid.

^{1}HNMR (360 MHz, DMSO-d6) δ: 10.63 (s, br, 1H, NH-1), 8.01 (s, 1H, H-vinyl), 7.71 - 7.74 (m, 2H), 7.63-7.65 (m,2H), 7.43-7.47 (m, 2H), 7.33-7.37 (m, 1H), 7.28 (dd, J = 1.66, 7.9Hz, 1H), 7.06 (d, J = 1.44Hz, 1H, H-7), 6.95 (dd, J = 0.97, 3.68Hz, 1H), 2.53 (s, 3H, CH_3). MS m/z (relative intensity, %): 318.3 (100, $[M+1]^+$).

Compound PI-016: 3-(3,5-Diethyl-1H-pyrrol-2-ylmethylene)-6-phenyl-1,3-dihydroindol-2-one

3,5-Diethyl-1H-pyrrole-2-carboxaldehyde (90.6 mg), 105 mg 6-phenyl-2-oxindole and 1 drop piperidine in 2 mL ethanol were heated to 95 °C and held overnight. The reaction mixture was cooled and the precipitate which formed was filtered, washed with cold ethanol and hexane and dried in a vacuum oven overnight to give 122 mg of the title compound (71%) as a red solid.

^{1}HNMR (360 MHz, DMSO-d6) δ: 13.51 (s, br, 1H, NH-1'), 10.83 (s, br, 1H, NH-1), 7.79 (d, J = 8.08Hz, 1H, H-4), 7.63 (dd, 2H), 7.6 (s, 1H, H-vinyl), 7,45 (t, 2H), 7.33 (7, 1H), 7.27 (dd, J = 1.5, 8.08Hz, 1H, H-5), 7.1 (d, J = 1.5Hz, 1H, H-7), 6.1 (d, J = 2.18Hz, 1H, H- 4'), 2.67-2.78 (m, 4H, 2xCH_2CH_3), 1.18-1.27 (m, 6H, 2xCH_2CH_3). MS m/z (relative intensity, %): 430.9 (25, $[M+1]^+$).

Compound PI-017: 3-(3,5-Dimethyl-1H-pyrrol-2-ylmethylene)-6-(4-methoxyphenyl)-1,3-dihydroindol-2-one

Tetrakis(triphenylphosphine)palladium (1 g) was added to a mixture of 5 g 4-methoxyphenylboronic acid, 6.6 g 5-bromo-2-fluoronitrobenzene and 30 mL of 2 M sodium carbonate in 50 mL toluene and 50 mL ethanol. The mixture was refluxed for 2 hours, concentrated, and the residue extracted twice with ethyl acetate. The ethyl acetate layer was washed with water and brine, dried, and concentrated to give a brown oily solid. The solid was chromatographed on silica gel using 5 % ethyl acetate in hexane as the eluent to give crude 4-fluoro-4'-methoxy-3-nitrobiphenyl as a pale yellow solid.

Dimethyl malonate (10 mL) was added dropwise to 2.0 g sodium hydride suspended in 60 mL dimethylsulfoxide. The mixture was heated to 100 °C, stirred at that temperature for 10 minutes and then cooled to room temperature. Crude 4-fluoro-2'-methoxy-3-nitrobiphenyl (5.2 g) in 50 mL dimethylsulfoxide was added and the mixture was again heated to 100 °C and stirred at that temperature for 2 hours. The reaction mixture was cooled, quenched with 300 mL saturated sodium chloride and extracted three times with ethyl acetate. The extracts were combined, washed with saturated ammonium chloride, water and brine, dried over anhydrous sodium sulfate and concentrated to give crude dimethyl 4'-methoxy-3-nitrobiphenyl-4-malonate as a yellow oil.

Crude 4'-methoxy-3-nitrobiphenyl-4-malonate was heated to 100 °C in 60 mL 6 N hydrochloric acid and stirred at that temperature for 15 hours after which the mixture was cooled to room temperature. The precipitate which formed was collected by filtration, washed with water and hexane, and dried to give 7.2 g of crude 4'-methoxy-3-nitrobiphenyl-4-acetic acid as a light tan solid.

Iron powder (3.6 g) was added in one portion to 7.2 g 4'-methoxy-3-nitrobiphenyl-4-acetic acid in 50 mL glacial acetic acid and the mixture was heated to 100 °C and stirred overnight at that temperature. The reaction mixture was then concentrated to dryness, sonicated in ethyl acetate and filtered to remove insoluble materials. The filtrate was washed twice with 1 N hydrochloric acid, then with brine, then dried over anhydrous sodium sulfate and concentrated to give 2.7 g (54 % yield based on 5-bromo-2-fluoronitrobenzene) of 6-(4-methoxyphenyl)-2-oxindole as a rose colored solid.

^{1}HNMR (360 MHz, DMSO-d6) δ: 10.38 (s, br, 1H, NH-1), 7.52 (d, J = 9Hz, 2H,), 7.23 (d, J = 7.3Hz, 1H, H-4), 7.14 (dd, J = 1.38, 7.3Hz, 1H, H-5), 7.0 (d, J = 9Hz, 2H), 6.96 (d, J = 1.38Hz, 1H, H-7), 3.78 (s, 3H, OCH_3), 3.47 (s, 2H, CH_2). MS m/z (relative intensity, %): 214.0 (100, $[M+1]^+$).

3,5-Dimethyl-1H-pyrrole-2-carboxaldehyde (74 mg), 120 mg 6-(4-methoxyphenyl)-2-oxindole and 1 drop piperidine in 2 mL ethanol were heated to 90 °C and held overnight at that temperature. The reaction mixture was then cooled and the precipitate which formed was filtered, washed with cold ethanol and hexane and dried in a vacuum oven overnight to give 87 mg of the title compound (50%) as a brown solid.

^{1}HNMR (360 MHz, DMSO-d6) δ: 13.31 (s, br, 1H, NH-1'), 10.79 (s, br, 1H, NH-1), 7.74 (d, J = 7.85Hz, 1H, H-4), 7.54-7.57 (m, 3H), 7.21 (dd, J = 1.71, 7.85Hz, 1H, H-5), 7.04 (d, J = 1.71Hz, 1H, H-7), 6.99-7.02 (m, 2H), 6.0 (d, J = 2.22Hz, 1H, H-4'), 3.79 (s, 3H, OCH_3), 2.32 (s, 3H, CH_3), 2.3 (s, 3H, CH_3). MS (APCI) m/z (relative intensity, %): 345.0 (100, $[M+1]^+$).

Compound PI-018: 3-(3,5-Diethyl-1H-pyrrol-2-ylmethylene)-6-(4-methoxyphenyl)-1,3-dihydroindol-2-one

3,5-Diethyl-1H-pyrrole-2-carboxaldehyde (91 mg), 120 mg 6-(4-methoxyphenyl)-2-oxindole and 1 drop piperidine in 2 mL ethanol were heated to 90 °C and held at that temperature for 4 hours. The reaction mixture was cooled and the precipitate which formed was filtered, washed with cold ethanol and hexane and dried in a vacuum oven overnight to give 90 mg of the title compound (48%) as a dark red solid.

^{1}HNMR (360 MHz, DMSO-d6) δ: 13.49 (s, br, 1H, NH-1'), 10.78 (s, br, 1H, NH-1), 7.74 (d, J = 8.03Hz, 1H, H-4), 7.55-7.57 (m, 3H), 7.21 (dd, J = 1.41, 8.03Hz, 1H, H-5), 7.05 (d, J = 1.41Hz, 1H, H-7), 7.01 (d, J = 9.02Hz, 2H, aromatic), 6.08 (d, J = 2.23Hz, 1H, H-4'), 3.79 (s, 3H, OCH_3), 2.66-2.77 (m, 4H, 2xCH_2CH_3), 1.18-1.27 (m, 6H, 2xCH_2CH_3). MS (APCI) m/z (relative intensity, %): 373 (100, $[M+1]^+$).

Compound PI-019: 3-(3H-Imidazol-4-ylmethylene)-6-(4-methoxyphenyl)-1,3-dihydroindol-2-one

3H-Imidazole-4-carboxaldehyde (58 mg), 120 mg 6-(4-methoxyphenyl)-2-oxindole and 1 drop piperidine in 2 mL ethanol were heated to 90 °C and held at that temperature for 4 hours. The reaction mixture was cooled and the precipitate which formed was filtered, washed with cold ethanol and hexane and dried in a vacuum oven overnight to give 85 mg of the title compound (54%) as a dark red solid.

^{1}HNMR (360 MHz, DMSO-d6) δ: 13.67 (s, br, 1H, NH-1'), 11.03 (s, br, 1H, NH-1), 8.01 (s, 1H), 7.83 (s, 1H), 6.96- 7.71 (m, 8H), 3.79 (s, 3H, OCH_3). MS (APCI) m/z (relative intensity, %): 318 (100, $[M+1]^+$).

Compound PI-020: 6-(4-Methoxyphenyl)-3-(4,5,6,7-tetrahydro-1H-indol-2-ylmethylene)-1,3-dihydro-indol-2-one

4,5,6,7-Tetrahydro-1H-indole-2-carboxaldehyde (90 mg), 120 mg 6-(4-methoxyphenyl)-2-oxindole and 1 drop piperidine in 2 mL ethanol were heated to 90 °C and held at that temperature for 4 hours. The reaction mixture was cooled and the precipitate which formed was filtered, washed with cold ethanol and hexane and dried in a vacuum oven overnight to give 92 mg of the title compound (50%) as an orange solid.

^{1}HNMR (360 MHz, DMSO-d6) δ: 13.07 (s, br, 1H, NH-1'), 10.8 (s, br, 1H, NH-1), 7.61 (d, J = 8.02Hz, 1H, H-4), 7.58 (s, 1H, H-vinyl), 7.56 (d, J = 8.83Hz, 2H), 7.21 (dd, J = 1.57, 8.02Hz, 1H, H-5), 7.04 (d, J = 1.57Hz, 1H, H-7), 7.01 (d, J = 8.83Hz, 2H), 6.58 (d, br, J = 1.05Hz,1H), 3.79 (s, 3H, OCH_3), 2.71 (t, J = 6.05Hz, 2H), 2.51 (t, J = 6.05Hz, 2H), 1.7 - 1.79 (m, 4H). MS (APCI) m/z (relative intensity, %): 371.0 (100, $[M+1]^+$).

Compound PI-021: 6-(4-Methoxyphenyl)-3-(3-methyl-4,5,6,7-tetrahydro-1H-indol-2-ylmethylene)-1,3-dihydroindol-2-one

3-Methyl-4,5,6,7-tetrahydro-1H-indole-2-carboxaldehyde (98 mg), 120 mg 6-(4-

methoxyphenyl)-2-oxindole and 1 drop piperidine in 2 mL ethanol were heated to 90 °C and held at that temperature overnight. The reaction mixture was cooled and the precipitate which formed was filtered, washed with cold ethanol and hexane and dried in a vacuum oven overnight to give 92 mg of the title compound (48%) as an orange solid.

^{1}HNMR (360 MHz, DMSO-d6) δ: 13.21 (s, br, 1H, NH-1'), 10.76 (s, br, 1H, NH-1), 7.73 (d, J = 7.87Hz, 1H, H-4), 7.56 (s, 1H, H-vinyl), 7.56 (d, J = 8.63Hz, 2H), 7.2 (dd, J = 1.7, 7.87Hz, 1H, H-5), 7.04 (d, J = 1.7Hz, 1H, H-7), 7.0 (d, J = 8.63Hz, 2H), 3.81 (s, 3H, OCH_3), 2.67 (t, J = 5.66Hz, 2H), 2.41 (t, J = 5.66Hz, 2H), 2.21 (s, 3H, CH_3), 1.7-1.78 (m, 4H). MS (APCI) m/z (relative intensity, %): 385.0 (100, $[M+1]^+$).

Compound PI-022: 6-(4-Methoxyphenyl)-3-(5-methylthiophen-2-ylmethylene)-1,3-dihydroindol-2-one

5-Methylthiophene-2-carboxaldehyde (76 mg), 120 mg 6-(4-methoxyphenyl)-2-oxindole and 1 drop piperidine in 2 mL ethanol were heated to 90 °C and held at that temperature overnight. The reaction mixture was cooled and the precipitate which formed was filtered, washed with cold ethanol and hexane and dried in a vacuum oven overnight to give 78 mg of the title compound (45%) as a gold solid.

^{1}HNMR (360 MHz, DMSO-d6) δ: 10.58 (s, br, 1H, NH-1), 7.96 (s, 1H, H-vinyl), 7.72 (d, J = 3.68Hz, 1H, H-thiophene), 7.67 (d, J = 7.98Hz, 1H, H-4), 7.57 (d, J = 8.71Hz, 2H), 7.2 (dd, J = 1.7, 7.87Hz, 1H, H-5), 7.01 (d, J = 1.47Hz, 1H, H-7), 7.01 (d, J = 8.71Hz, 2H), 6.94 (d, J = 3.68Hz, 1H, H-thiophene), 3.79 (s, 3H, OCH_3), 2.53 (s, 3H, CH_3). MS (APCI) m/z (relative intensity, %): 347.0 (100, $[M+1]^+$).

Compound PI-023: 3-{2-[6-(3-Methoxyphenyl)-2-oxo-1,2-dihydroindol-3-ylidenemethyl]-4,5,6,7-tetrahydro-1H-indol-3-yl}-propionic acid

1-(Morpholin-4-yl)cyclohexene (300 g), 214 g triethylamine and 1400 mL dichloromethane were refluxed for 15 minutes and then cooled in a water bath to 15 – 20 °C. Ethyl succinyl chloride (266 g) dissolved in 500 mL dichloromethane was added over

30 minutes. The mixture was refluxed for 30 minutes and cooled to room temperature in a water bath. The solid which formed was collected by vacuum filtration, washed with 100 mL of dichloromethane and discarded. The filtrate was returned to the flask and the solvent removed by distillation to give 454 g of crude 4-(2-morpholin-4-yl-cyclohex-1-enyl)-4-oxobutyric acid ethyl ester as an oil.

Crude 4-(2-morpholin-4-yl-cyclohex-1-enyl)-4-oxobutyric acid ethyl ester (454 g), 398 g diethyl aminomalonate hydrochloride, 162 g sodium acetate and 350 mL glacial acetic acid were heated to 108 °C over 30 minutes. The mixture was stirred at 100 – 108 °C for 2 hours and then cooled to about 50 °C in a water bath. Water (2500 mL) and 700 mL ethyl acetate were added. The ethyl acetate layer was separated and washed three times with brine, twice with saturated sodium bicarbonate, once again with brine and then dried over anhydrous sodium sulfate and rotary evaporated to give 494 g (105 % yield) of crude 3-(2-ethoxycarbonyl-ethyl)-4,5,6,7-tetrahydro-1H-indole-2-carboxylic acid ethyl ester as an oil. The crude product was chromatographed on a silica gel column using ethyl acetate:hexane 1:10 as eluent to give 122.1 g of pure 3-(2-ethoxycarbonyl-ethyl)-4,5,6,7-tetrahydro-1H-indole-2-carboxylic acid ethyl ester as a low melting solid.

^{1}HNMR (d6-DMSO) δ: 11.0 (s, 1H, pyrrole NH), 4.2, 4.0(2xt, 4H, 2xC(O)CH_2), 2.8, 2.4(2xt, 4H, -CH_2CH_2C(O)-), 2.4(m, 4H, -CH_2-, -CH_2-), 1.7(m, 4H, - CH_2CH_2-).

Purified 3-(2-ethoxycarbonylethyl)-4,5,6,7-tetrahydro-1H-indole-2-carboxylic acid ethyl ester (122.1 g) and 328 mL 5 N sodium hydroxide were refluxed for 80 minutes. The heat was turned off and 165 mL of 10 N hydrochloric acid was cautiously added via a dropping funnel through the reflux condenser while the mixture was being vigorously stirred. The addition was continued until the pH was 2-3. The mixture was cooled in an ice bath upon which the oil that had formed solidified. The solid was collected by vacuum filtration, washed 3 times with water and dried under vacuum at 50 – 60 °C to give 54.9 g (71% yield) of 3-(4,5,6,7-tetrahydro-1H-indol-3-yl)propionic acid as a dark brown solid.

^{1}HNMR (d6-DMSO) δ: 13.1(s, 1H, pyrrole NH), 11.8(br s, 1H, COOH), 9.8(s, 1H, CH), 2.5, 2.3(2xt, 4H, - CH_2CH_2C(O)-), 2.4(m, 4H, - CH_2-, - CH_2-), 1.7(m, 4H, - CH_2CH_2-).

3-(2-Formyl-4, 5, 6, 7-tetrahydro-1H-indol-3-yl)-propionic acid (95 mg), 103 mg 6-(3-methoxyphenyl)-2-oxindole and 1 drop piperidine in 2 mL ethanol were heated to 90

°C and held at that temperature overnight. The reaction mixture was cooled and concentrated. The residue was suspended in 6 N hydrochloric acid and the precipitate which formed was filtered, washed with water until the pH of the wash was approximately 6 and then dried in a vacuum oven overnight to give 156 mg of the title compound (82%) as a brown solid.

^{1}HNMR (360 MHz, DMSO-d6) δ: 13.26 (s, br, 1H, NH-1'), 10.79 (s, br, 1H, NH-1), 7.72 (d, J = 8.1Hz, 1H, H-4), 7.64 (s, 1H, H-vinyl), 7.27 (dd, J = 1.47, 8.1Hz, 1H, H-5), 6.89-7.38 (m, 5H), 3.81 (s, 3H, OCH_3), 2.89- 2.93 (m, 2H), 2.66-2.68 (m, 2H), 2.38-2.43 (t, 4H), 1.7-1.78 (m, 4H). MS m/z (relative intensity, %): 443.2 (50, $[M+1]^+$).

Compound PI-024: 3-{2-[6-(4-Methoxyphenyl)-2-oxo-1,2-dihydroindol-3-ylidenemethyl]-4,5,6,7-tetrahydro-1H-indol-3-yl}-propionic acid

3-(2-Formyl-4,5,6,7-tetrahydro-1H-indol-3-yl)-propionic acid (95 mg), 103 mg 6-(4-methoxyphenyl)-2-oxindole and 1 drop piperidine in 2 mL ethanol were heated to 90 °C and held at that temperature overnight. The reaction mixture was cooled and concentrated. The residue was suspended in 6 N hydrochloric acid and the precipitate which formed was filtered, washed with water until the pH of the wash was approximately 6 and then dried in a vacuum oven overnight to give 57 mg of the title compound (30%) as a brown solid.

^{1}HNMR (360 MHz, DMSO-d6) δ: 13.24 (s, br, 1H, NH-1'), 11.61 (s, br, 1H, COOH), 10.76 (s, br, 1H, NH-1), 7.7 (d, J = 8.1Hz, 1H, H-4), 7.61 (s, 1H, H-vinyl), 7.56 (d, J = 8.81Hz, 2H), 7.21 (d, J = 1.47, 8.1Hz, 1H, H-5), 7.04 (d, J = 1.47Hz, 1H, H-7), 7.01 (d, J = 8.81Hz, 2H), 3.79 (s, 3H, OCH_3) , 2.89-2.93 (m, 2H), 2.66-2.67 (m, 2H), 2.4-2.46 (m, 4H), 1.6-1.78 (m, 4H). MS m/z (relative intensity, %): 441.2 (50, $[M-1]^-$).

Compound PI-025: 3-[2-(2-Oxo-6-phenyl-1,2-dihydroindol-3-ylidenemethyl)-4,5,6,7-tetrahydro-1H-indol-3-yl]-propionic acid

3-(2-Formyl-4,5,6,7-tetrahydro-1H-indol-3-yl)-propionic acid (95 mg), 90 mg 6-

phenyl-2-oxindole and 1 drop piperidine in 2 mL ethanol were heated to 90 °C and held at that temperature for 4 hours. The reaction mixture was then cooled and concentrated. The residue was acidified with 6 N hydrochloric acid and the precipitate which formed was filtered, washed with water until the pH of the wash was about 6 and then dried in a vacuum oven overnight to give 59 mg of the title compound (31%) as a brown solid.

^{1}HNMR (360 MHz, DMSO-d6) δ: 13.26 (s, br, 1H, NH-1'), 12.06 (s, br, 1H, COOH), 10.8 (s, br, 1H, NH-1), 7.74 (d, J = 8.1Hz, 1H, H-4), 7.66 (s, 1H, H-vinyl), 7.26-7.64 (m, 6H), 7.1 (s, br, 1H), 2.9- 2.94 (t, 2H), 2.66-2.68 (m, 2H), 2.41-2.46 (m, 4H), 1.73-1.76 (m, 4H). MS m/z (relative intensity, %): 411.2 (50, $[M-1]^-$).

Compound PI-026: 3-{2-[6-(2-Methoxyphenyl)-2-oxo-1,2-dihydroindol-3-ylidenemethyl]-4,5,6,7-tetrahydro-1H-indol-3-yl}-propionic acid

Tetrakis(triphenylphosphine)palladium (1 g) was added to a mixture of 5 g 2-methoxyphenylboronic acid, 6.6 g 5-bromo-2-fluoronitrobenzene and 30 mL 2 M sodium carbonate in 50 mL toluene and 50 mL ethanol. The mixture was refluxed for 2 hours, concentrated, and the residue extracted twice with ethyl acetate. The ethyl acetate extracts were combined, washed with water and brine, dried, and concentrated to give crude 4-fluoro-2'-methoxy-3-nitrobiphenyl as a dark green oil which solidified on standing.

Dimethyl malonate (14 mL) was added dropwise to 2.9 g sodium hydride suspended in 50 mL of dimethylsulfoxide. The mixture was heated to 100 °C and stirred at that temperature for 15 minutes. The reaction was then cooled to room temperature. Crude 4-fluoro-2'-methoxy-3-nitrobiphenyl in 60 mL dimethylsulfoxide was added and the mixture was again heated to 100 °C and stirred for 2 more hours. The reaction mixture was then cooled and quenched with 300 mL saturated sodium chloride and extracted twice with ethyl acetate. The extracts were combined, washed with saturated ammonium chloride, water, and brine, dried over anhydrous sodium sulfate and concentrated to give crude dimethyl 2'-methoxy-3-nitrobiphenyl-4-malonate as a yellow oil.

Crude 2'-methoxy-3-nitrobiphenyl-4-malonate was heated to 100 °C in 50 mL 6 N hydrochloric acid and held at that temperature with stirring for 24 hours. The mixture was

then cooled and the precipitate which formed was filtered, washed with water and hexane and then dried to give 9.8 g of 2'-methoxy-3-nitrobiphenyl-4-acetic acid as a light tan solid.

Iron powder (5 g) was added in one portion to 9.8 g 2'methoxy-3-nitrobiphenyl-4-acetic acid in 50 mL glacial acetic acid and the mixture was heated to 100 °C and stirred at that temperature for 3 hours. The reaction mixture was then concentrated to dryness, sonicated in ethyl acetate and filtered to remove insoluble materials. The filtrate was washed twice with 1 N hydrochloric acid, then with water, then with brine. The filtrate was then dried over anhydrous sodium sulfate and concentrated. The residue obtained was chromatographed on silica gel using ethyl acetate:hexane 1:2 as eluent to give 5.4 g (69 % yield based on 5-bromo-2-fluoronitrobenzene) of 6-(2-methoxyphenyl)-2-oxindole as a rose colored solid.

^{1}HNMR (360 MHz, DMSO-d6) δ: 10.32 (s, br, 1H, NH), 7.29- 7.34 (m, 1H), 7.19-7.25 (m, 2H), 7.08 (d, J = 8Hz, 1H, H-4), 6.97-7.02 (m, 2H), 6.91 (d, J = 1.05Hz, 1H, H-7), 3.8 (s, 3H, OCH_3), 3.47 (s, 2H, CH_2). MS m/z (relative intensity, %): 239.8 (100, $[M+1]^+$).

3-(2-Formyl-4,5,6,7-tetrahydro-1H-indol-3-yl)-propionic acid (95 mg), 103 mg 6-(2-methoxyphenyl)-2-oxindole and 1 drop piperidine in 2 mL ethanol were heated to 90 °C and held at that temperature for 4 hours. The reaction mixture was then cooled and concentrated. The residue was acidified with 6 N hydrochloric acid and the precipitate which formed was filtered, washed with water until the pH of the washes was about 6 and then dried in a vacuum oven overnight to give 67 mg of the title compound (35%) as a brown solid.

^{1}HNMR (360 MHz, DMSO-d6) δ: 13.26 (s, br, 1H, NH-1'), 12.06 (s, br, 1H, COOH), 10.7 (s, br, 1H, NH-1), 7.67 (d, J = 7.72Hz, 1H, H-4), 7.61 (s, 1H, H-vinyl), 7.27-7.34 (m, 2H), 7.01-7.1 (m, 3H), 6.99 (d, J = 1.1Hz, 1H, H-7), 3.76 (s, 3H, OCH_3) , 2.89-2.93 (t, 2H), 2.66-2.69 (m, 2H), 2.4-2.46 (m, 4H), 1.71 - 1.78 (m, 4H). MS m/z (relative intensity, %): 441.2 (50, $[M-1]^-$).

Compound PI-027: 3,5-Dimethyl-1H-pyrrol-2-ylmethylene)-6-(2-methoxyphenyl)-1,3-dihydroindol-2-one

3,5-Dimethyl-1H-pyrrole-2-carboxaldehyde (150 mg), 239 mg 6-(2-methoxyphenyl)-2-oxindole and 1 drop piperidine in 2 mL ethanol were heated to 90 °C and held at that temperature for 4 hours. The reaction mixture was then cooled and the precipitate which formed was filtered, washed with cold ethanol and hexane and dried in a vacuum oven overnight to give 268 mg of the title compound (78%) as a brown solid.

^{1}HNMR (360 MHz, DMSO-d6) δ: 13.33 (s, br, 1H, NH-1'), 10.73 (s, br, 1H, NH-1), 7.77 (d, J = 8.1Hz, 1H, H-4), 7.55 (s, 1H, H-vinyl), 7.28-7.34 (m, 2H), 6.99-7.1 (m, 4H), 6.0 (d, J = 2.07Hz, 1H, H- 4'), 3.76 (s, 3H, OCH_3), 2.32 (s, 3H, CH_3), 2.3 (s, 3H, CH_3). MS (APCI) m/z (relative intensity, %): 345.0 (100, $[M+1]^+$).

Compound PI-028: 3,5-Diethyl-1H-pyrrol-2-ylmethylene)-6-(2-methoxyphenyl)-1,3-dihydroindol-2-one

3,5-Diethyl-1H-pyrrole-2-carboxaldehyde (181 mg), 239 mg 6-(2-methoxyphenyl)-2-oxindole and 1 drop piperidine in 2 mL ethanol were heated to 90 °C and held at that temperature for 4 hours. The reaction mixture was then cooled and the precipitate which formed was filtered, washed with cold ethanol and hexane and dried in a vacuum oven overnight to give 303 mg of the title compound (81%) as a brown solid.

^{1}HNMR (360 MHz, DMSO-d6) δ: 13.51 (s, br, 1H, NH-1'), 10.72 (s, br, 1H, NH-1), 7.71 (d, J = 7.73Hz, 1H, H-4), 7.57 (s, 1H, H-vinyl), 7.28-7.34 (m, 2H), 6.99-7.1 (m, 4H), 6.08 (d, J = 2.21Hz, 1H, H- 4'), 3.76 (s, 3H, OCH_3), 2.66-2.77 (m, 4H, CH_2CH_3), 1.18-1.27 (m, 6H, CH_2CH_3). MS m/z (relative intensity, %): 373.5 (100, $[M+1]^+$).

Compound PI-029: 3-(3H-Imidazol-4-ylmethylene)-6-(2-methoxyphenyl)-1,3-dihydroindol-2-one

3H-Imidazole-4-carboxaldehyde (115 mg), 239 mg 6-(2-methoxyphenyl)-2-oxindole and 1 drop piperidine in 2 mL ethanol were heated to 90 °C and held at that

temperature for 4 hours. The reaction mixture was then cooled and the precipitate which formed was filtered, washed with cold ethanol and hexane and dried in a vacuum oven overnight to give 257 mg of the title compound (81%) as a yellow solid.

^{1}HNMR (360 MHz, DMSO-d6) δ: 13.71 (s, br, 1H, NH-1'), 10.96 (s, br, 1H, NH-1), 8.0 (s, br, 1H), 7.82 (s, br,1H). 7.64-7.68 (m, 2H), 7.28-7.36 (m, 2H), 7.09-7.12 (m, 2H), 7.0-7.04 (m, 2H), 3.77 (s, 3H, OCH_3). MS m/z (relative intensity, %): 318.5 (100, $[M+1]^+$).

Compound PI-030: 6-(2-Methoxyphenyl)-3-(4,5,6,7-tetrahydro-1H-indol-2-ylmethylene)-1,3-dihydroindol-2-one

4,5,6,7-Tetrahydro-1H-indole-2-carboxaldehyde (180 mg), 239 mg 6-(2-methoxyphenyl)-2-oxindole and 1 drop piperidine in 2 mL ethanol were heated to 90 °C and held at that temperature for 4 hours. The reaction mixture was then cooled and the precipitate which formed was filtered, washed with cold ethanol and hexane and dried in a vacuum oven overnight to give 315 mg of the title compound (85%) as an orange solid.

^{1}HNMR (360 MHz, DMSO-d6) δ: 13.11 (s, br, 1H, NH-1'), 10.74 (s, br, 1H, NH-1), 7.58 (s, 1H, H-vinyl), 6.99-7.59 (m, 7H), 6.58 (s, br, 1H, H-3'), 3.76 (s, 3H, OCH_3), 2.71 (t, 2H), 2.5-2.53 (t, 2H), 1.7-1.79 (m, 4H). MS m/z (relative intensity, %): 371.5 (100, $[M+1]^+$).

Compound PI-031: 6-(2-Methoxyphenyl)-3-(3-methyl-4,5,6,7-tetrahydro-1H-indol-2-ylmethylene)-1,3-dihydroindol-2-one

3-Methyl-4,5,6,7-tetrahydro-1H-indole-2-carboxaldehyde (195 mg), 239 mg 6-(2-methoxyphenyl)-2-oxindole and 1 drop piperidine in 2 mL ethanol were heated to 90 °C and held at that temperature for 4 hours. The reaction mixture was then cooled and the precipitate which formed was filtered, washed with cold ethanol and hexane and dried in a vacuum oven overnight to give 317 mg of the title compound (82%) as an orange solid.

^{1}HNMR (360 MHz, DMSO-d6) δ: 13.24 (s, br, 1H, NH-1'), 10.68 (s, br, 1H, NH-1), 7.7 (d, J = 7.62Hz, 1H, H-4), 7.57 (s, 1H, H-vinyl), 6.99-7.33 (m, 6H), 3.76 (s, 3H,

OCH_3), 2.67 (t, 2H,), 2.41 (t, 2H,), 1.71-1.78 (m, 4H). MS m/z (relative intensity, %): 385.5 (100, $[M+1]^+$).

Compound PI-032: 3-(3,5-Diisopropyl-4-methoxybenzylidene)-6-(3-methoxyphenyl)-1,3-dihydroindol-2-one

To a stirred suspension of 2,6-diisopropylphenol (10 g) and potassium carbonate (9.2 g) in dimethylformamide (50 mL) at room temperature was slowly added 9.5 g iodomethane. The mixture was stirred at room temperature overnight. The mixture was then poured into water. The precipitate which formed was collected by vacuum filtration and washed with water. The precipitate was then vacuum dried to give 10.3 g of 1,3-diisopropyl-2-methoxybenzene.

To a stirred suspension of 7.3 g Vilsmeier reagent in 100 mL dichloromethane at 0 °C was added 10 g 1,3-diisopropyl-2-methoxybenzene in portions so as to keep the reaction temperature below 10 °C. The mixture was then refluxed for 4 hours and then cooled to 5 °C. Water (100 mL) was added and the aqueous layer separated and saved. The organic layer was extracted with 100 mL of water and the aqueous extracts were combined and cooled to 5 °C. The combined aqueous extracts were then stirred while 100 mL 2.5 N sodium hydroxide was slowly added. When all of the sodium hydroxide had been added the mixture was stirred for an additional hour, the temperature being held at about 5 °C. The aqueous solution was then extracted with ethyl acetate and the extract washed with brine, dried with anhydrous sodium sulfate and concentrated. The residue was chromatographed on a silica gel column to give 8.02 g of 1,3-diisopropyl-4-methoxybenzaldehyde (70%) as a pale yellow oil.

^{1}HNMR (360 MHz, DMSO-d6) δ: 9.92 (s, 1H, CHO), 7.69 (s, 2H, H-Ar), 3.23-3.34 (m, J = 6.9Hz, 2H), 1.2 (d, J = 6.9Hz, 12H). MS m/z (relative intensity, %): 221.2 (30, $[M+1]^+$).

1,3-diisopropyl-4-methoxybenzaldehyde (264 mg), 239 mg 6-(3-methoxyphenyl)-2-oxindole and 1 drop piperidine in 2 mL ethanol were heated to 90 °C and held at that temperature for 4 hours. The reaction mixture was then cooled and the precipitate which formed was filtered, washed with cold ethanol and hexane and dried in a vacuum oven

overnight to give the title compound as an orange colored solid.

^{1}HNMR (360 MHz, DMSO-d6) δ: 10.61 (s, br, 1H, NH), 7.68 (d, J = 8.13Hz, 1H), 7.61 (s, 1H, H-vinyl), 7.53 (s, 2H), 7.37 (t, J = 7.8Hz, 1H), 7.14-7.2 (m, 3H), 7.06 (d, J = 1.55Hz, 1H), 6.94 (dd, J = 2.7, 7.9HZ, 1H), 3.81 (s, 3H, OCH_3), 3.75 (s, 3H, OCH_3), 3.25-3.35 (m, 2H), 1.23(d, J = 7 Hz, 12H). MS (APCI) m/z (relative intensity, %): 442.1 (100, $[M+1]^+$).

Compound PI-033: 3-(3,5-Diisopropyl-4-methoxybenzylidene)-6-(3-ethoxyphenyl)-1,3-dihydroindol-2-one

1,3-diisopropyl-4-methoxybenzaldehyde (264 mg), 253 mg 6-(3-ethoxyphenyl)-2-oxindole and 1 drop piperidine in 2 mL ethanol were heated to 90 °C and held at that temperature for 4 hours. The reaction mixture was then cooled and the precipitate which formed was filtered, washed with cold ethanol and hexane and dried in a vacuum oven overnight to give the title compound as a brown solid.

^{1}HNMR (360 MHz, DMSO-d6) δ: 10.60 (s, br, 1H, NH), 7.68 (d, J = 8.1Hz, 1H), 7.61 (s, 1H, H-vinyl), 7.53 (s, 2H), 7.37 (t, J = 7.8Hz, 1H), 7.14-7.19 (m, 3H), 7.11 (d, J = 1.58Hz, 1H), 6.92-6.96 (m, 1H), 4.09 (q, J = 7.2Hz, 2H, OCH_2CH_3), 3.81 (s, 3H, OCH_3), 3.25-3.35 (m, 2H), 1.33 (t, J = 7.2Hz, 3H, OCH_2CH_3), 1.23 (d, J = 7 Hz, 12H). MS (APCI) m/z (relative intensity, %): 456.2 (100, $[M+1]^+$).

Compound PI-034: 3-(3,5-Diisopropyl-4-methoxybenzylidene)-6-phenyl-1,3-dihydroindol-2-one

1,3-diisopropyl-4-methoxybenzaldehyde (264 mg), 239 mg 6-phenyl-2-oxindole and 1 drop piperidine in 2 mL ethanol were heated to 90 °C and held at that temperature for 4 hours. The reaction mixture was then cooled and the precipitate which formed was filtered, washed with cold ethanol and hexane and dried in a vacuum oven overnight to give the title compound as a golden brown solid.

^{1}HNMR (360 MHz, DMSO-d6) δ: 10.63 (s, br, 1H, NH), 7.69 (d, J = 8.13Hz, 1H), 7.61-7.64 (m, 3H), 7.53 (s, 2H), 7.44-7.48 (m, 2H), 7.35-7.39 (m, 1H), 7.16 (dd, J = 1.74,

8.08Hz, 1H), 7.11 (d, J = 1.44Hz, 1H), 3.75 (s, 3H, OCH_3), 3.25-3.35 (m, 2H), 1.23 (d, J=ZH_3 12H). MS (APCI) m/z (relative intensity, %): 412.1 (100, $[M+1]^+$).

ASSAY PROCEDURES

The following *in vitro* assays may be used to determine the level of activity and effect of the different compounds of the present invention on one or more of the PKS. Similar assays can be designed along the same lines for any PK using techniques well known in the art.

The cellular/catalytic assays described herein are performed in an ELISA format. The general procedure is as follows: a compound is introduced to cells expressing the test kinase, either naturally or recombinantly, for some period of time after which, if the test kinase is a ligand known to activate the receptor's activity is added. The cells are lysed and the lysate is transferred to the wells of an ELISA plate previously coated with a specific antibody recognizing the substrate of the enzymatic phosphorylation reaction. Non-substrate components of the cell lysate are washed away and the amount of phoshorylation on the substrate is detected with an antibody specifically recognizing phoshotyrosine compared with control cells that were not contacted with a test compound.

The cellular/biologic assays described herein measure the amount of DNA made in response to activation of a test kinase, which is a general measure of a proliferative response. The general procedure for this assay is as follows: a compound is introduced to cells expressing the test kinase, either naturally or recombinantly, for some period of time after which, if the test kinase is a receptor, a ligand known to activate the receptor's activity is added. After incubation at least overnight, a DNA labeling reagent such as Bromodeoxy-uridine (BrdU) or 3H-thymidine is added. The amount of labeled DNA is detected with either an anti-BrdU antibody or by measuring radioactivity and is compared to control cells not contacted with a test compound.

EXAMPLE 6: ASSAY MEASURING THE EGF RECEPTOR KINASE ACTIVITY

EGF Receptor kinase activity in cells genetically engineered to express human

EGF-R is measured as described below:

Materials and Reagents.

The following materials andreagents are used:

a. EGF Ligand: stock concentration = 16.5 μM; EGF 201, TOYOBO, Co., Ltd. Japan.

b. 05-101 (UBI) (a monoclonal antibody recognizing an EGFR extracellular domain).

c. Anti-phosphotyosine antibody (anti-Ptyr) (polyclonal).

d. Detection antibody: Goat anti-rabbit lgG horse radish peroxidase conjugate, TAGO, Inc., Burlingame, CA.

e. TBST buffer:

Tris-HCl, pH 7	50 mM
NaCl	150 mM
Triton X-100	0.1

f. HNTG SX stock:

HEPES	0.1 M
NaCl	0.75 M
Glycerol	50
Triton X-100	1.0%

g. ABTS stock:

Citric Acid	100 mM
NA_2HPO_4	250 mM
HCl, conc.	4.0 pH
ABTS*	0.5 mg/mL

Keep solution in dark at 4 °C until used.

h. Stock reagents of:

EDTA	100 mM pH 7.0
Na_3VO_4	0.5 M
$Na_4(P_2O_7)$	0.2 M

Procedure

The following protocol is used:

A. Pre-coat ELISA Plate

1. Coat ELISA plates (Corning, 96 well, Cat. #25805-96) with 05-101 antibody at 0.5 µg per well in PBS, 150 µL final volume/well, and store overnight at 4 °C. Coated plates are good for up to 10 days when stored at 4 °C.

2. On day of use, remove coating buffer and replace with blocking buffer (5% Carnation Instant NonFat Dry Milk in PBS). Incubate the plate, shaking, at room temperature (about 23 °C to 25 °C) for 30 minutes. Just prior to use, remove blocking buffer and wash plate 4 times with TBST buffer.

B. Seeding Cells

1. NIH 3T3/C7 cell line (Honegger, et al., Cell 51:199-209, 1987) can be used for this assay.

2. Choose dishes having 80-90% confluence for the experiment. Trypsinize cells and stop reaction by adding 10% CS DMEM medium. Suspend cells in DMEM medium (10% CS DMEM medium) and centrifuge once at 1000 rpm, and once at room temperature for 5 minutes.

3. Resuspend cells in seeding medium (DMEM, 0.5% bovine serum), and count the cells using trypan blue. Viability above 90% is acceptable. Seed cells in DMEM medium (0.5% bovine serum) at a density of 10,000 cells per well, 100 µL per well, in a 96 well microtiter plate. Incubate seeded cells in 5% CO_2 at 37 °C for about 40 hours.

C. Assay Procedures.

1. Check seeded cells for contamination using an inverted microscope. Dilute drug stock (10 mg/mL in DMSO) 1:10 in DMEM medium, then transfer 5 µL to a test well for a final drug dilution of 1:200 and a final DMSO concentration of 1%. Control wells receive DMSO alone. Incubate in 5% CO_2 at 37 °C for one hour.

2. Prepare EGF ligand: dilute stock EGF in DMEM so that upon transfer of 10 µL dilute EGF (1:12 dilution), 25 nM final concentration is attained.

3. Prepare fresh 10 mL HNTG* sufficient for 100 µL per well wherein HNTG* comprises: HNTG stock (2.0 mL), milli-Q H_2O (7.3 mL), EDTA, 100 mM, pH 7.0 (0.5 mL), Na_3VO_4 0.5 M (0.1 mL) and $Na_4(P_2O_7)$, 0.2 M (0.1 mL).

4. Place on ice.

5. After two hours incubation with drug, add prepared EGF ligand to cells, 10 µL per well, to yield a final concentration of 25 nM. Control wells receive DMEM alone. Incubate, shaking, at room temperature, for 5 minutes.

6. Remove drug, EGF, and DMEM. Wash cells twice with PBS. Transfer HNTG to cells, 100 µL per well. Place on ice for 5 minutes. Meanwhile, remove blocking buffer from other ELISA plate and wash with TBST as described above.

7. With a pipette tip securely fitted to a micropipettor, scrape cells from plate and homogenize cell material by repeatedly aspirating and dispensing the HNTG* lysis buffer. Transfer lysate to a coated, blocked, and washed ELISA plate. Incubate shaking at room temperature for one hour.

8. Remove lysate and wash 4 times with TBST. Transfer freshly diluted anti-Ptyr antibody to ELISA plate at 100 µL per well. Incubate shaking at room temperature for 30 minutes in the presence of the anti-Ptyr antiserum (1:3000 dilution in TBST).

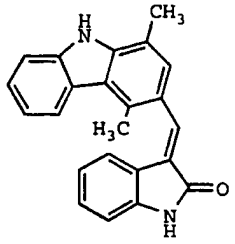
9. Remove the anti-Ptyr antibody and wash 4 times with TBST. Transfer the freshly diluted TAGO 30 anti-rabbit IgG antibody to the ELISA plate at 100 µL per well. Incubate shaking at room temperature for 30 minutes (anti-rabbit IgG antibody: 1:3000 dilution in TBST).

10. Remove detection antibody and wash 4 times with TBST. Transfer freshly prepared ABTS/H_2O_2 solution to ELISA plate, 100 µL per well. Incubate at room temperature for 20 minutes. ABTS/H_2O_2 solution: 1.2 µL 30% H_2O_2 in 10 mL ABTS stock.

11. Stop reaction by adding 50 µL 5N H_2SO_4 (optional), and determine O.D. at 410 rpm.

12. The maximal phosphotyrosine signal is determined by subtracting the value of the negative controls from the positive controls. The percent inhibition of phosphotyrosine content for extract-containing wells is then calculated, after subtraction of the negative controls.

The IC_{50} values measured in the EGF receptor phosphorylation assay for the compound of formula XXIV was found to be > 100 μM.



(XXIV)

EXAMPLE 7: ASSAY MEASURING THE IGF-1 RECEPTOR KINASE ACTIVITY

The following protocol is used to measure phosphotyrosine level on IGF-1 receptor, which indicates IGF-1 receptor tyrosine kinase activity.

Materials And Reagents

The following materials and reagents are used:

a. The cell line used in this assay is 3T3-L1/IGF-1R, a cell line genetically engineered to overexpresses IGF-1 receptor.

b. NIH3T3/IGF-1R is grown in an incubator with 5% CO_2 at 37 °C. The growth media is DMEM + 10% FBS (heat inactivated)+ 2 mM L-glutamine.

c. Affinity purified anti-IGF-1R antibody 17-69.

d. D-PBS:

KH_2PO_4	0.20 g/L
K_2HPO_4	2.16 g/L
KCl	0.20 g/L
NaCl	8.00 g/L (pH 7.2)

e. Blocking Buffer: TBST plus 5% Milk (Carnation Instant Non-Fat Dry Milk).

f. TBST buffer:

Tris-HCl	50 mM
NaCl	150mM (pH 7.2/HCl 1 N)
Triton X-100	0.1%

Stock solution of TBS (10X) is prepared, and Triton X-100 is added to the buffer during dilution.

g. HNTG buffer:

HEPES	20 mM
NaCl	150 mM (pH 7.2/HCl 1 N)
Glycerol	10%
Triton X-100	0.2%

Stock solution (5X) is prepared and kept at 4 °C.

h. EDTA/HCl: 0.5 M pH 7.0 (NaOH) as 100X stock.

i. Na_3VO_4: 0.5 M as 100X stock and aliquots are kept in -80 °C.

j. $Na_4P_2O_7$: 0.2 M as 100X stock.

k. Insulin-like growth factor-1 from Promega (Cat# G5111).

l. Rabbit polyclonal anti-phosphotyrosine antiserum.

m. Goat anti-rabbit IgG, POD conjugate (detection antibody), Tago (Cat. No. 4520, Lot No. 1802): Tago, Inc., Burlingame, CA.

n. ABTS (2.2'-azinobis(3-ethylbenzthiazolinesulfonic acid)) solution:

Citric acid	100 mM
a_2HPO_4	250 mM (pH 4.0/1 N HCl)
ABTS	0.5 mg/mL

ABTS solution should be kept in dark and 4 °C. The solution should be discarded when it turns green.

o. Hydrogen Peroxide: 30% solution is kept in the dark at 4 °C.

Procedure

All the following steps are conducted at room temperature unless it is specifically indicated. All ELISA plate washings are performed by rinsing the plate with tap water three times, followed by one TBST rinse. Pat plate dry with paper towels.

A. Cell Seeding:

1. The cells, grown in tissue culture dish (Corning 25020-100) to 80-90%

confluence, are harvested with Trypsin-EDTA (0.25%, 0.5 mL/D-100, GIBCO).

2. Resuspend the cells in fresh DMEM + 10% FBS + 2mM L-Glutamine, and transfer to 96 - well tissue culture plate (Corning, 250806-96) at 20,000 cells/well (100 µL/well). Incubate for 1 day then replace medium to serum-free medium (90/µL) and incubate in 5% CO_2 and 37 °C overnight.

B. ELISA Plate Coating and Blocking:

1. Coat the ELISA plate (Corning 25805-96) with Anti-IGF-lR Antibody at 0.5 µg/well in 100 µL PBS at least 2 hours.

2. Remove the coating solution, and replace with 100 µL Blocking Buffer, and shake for 30 minutes. Remove the blocking buffer and wash the plate just before adding lysate.

C. Assay Procedures:

1. The drugs are tested in serum-free condition.

2. Dilute drug stock (in 100% DMSO) 1:10 with DMEM in 96-well poly-propylene plate, and transfer 10 µL/well of this solution to the cells to achieve final drug dilution 1:100, and final DMSO concentration of 1.0%. Incubate the cells in 5% $C0_2$ at 37 °C for 2 hours.

3. Prepare fresh cell lysis buffer (HNTG*)

HNTG	2 mL
EDTA	0.1 mL
Na_3VO_4	0.1 mL
$Na_4(P_2O_7)$	0.1 mL
H_2O	7.3 mL

4. After drug incubation for two hours, transfer 10 µL/well of 2OOnM IGF-1 Ligand in PBS to the cells (Final Conc. = 20 nM), and incubate at 5% CO_2 at 37 °C for 10 minutes.

5. Remove media and add 100µL/well HNTG* and shake for 10 minutes. Look at cells under microscope to see if they are adequately lysed.

6. Use a 12-channel pipette to scrape the cells from the plate, and homogenize the lysate by repeat aspiration and dispense. Transfer all the lysate to the antibody coated

ELISA plate, and shake for 1 hour.

7. Remove the lysate, wash the plate, transfer anti-pTyr (1:3,000 with TBST) 100 μL/well, and shake for 30 minutes.

8. Remove anti-pTyr, wash the plate, transfer Tago (1:3,000 with TBST) 100 μL/well, and shake for 30 minutes.

9. Remove detection antibody, wash the plate, and transfer fresh ABTS/H_2O_2 to 10 mL ABTS) 100 μL/well to the plate to start color developments.

10. Measure OD at 410 nm with a reference wavelength of 630 nm in Dynatec MR5000.

The IC_{50} value measured in the IGF-1 receptor phosphorylation assay for the compound of formula XXIV (Example 6) was found to be 8.52 μM.

EXAMPLE 8: ASSAY MEASURING THE PDGF RECEPTOR KINASE ACTIVITY

All cell culture media, glutamine, and fetal bovine serum were purchased from Gibco Life Technologies (Grand Island, NY) unless otherwise specified. All cells were grown in a humid atmosphere of 90-95% air and 5-10% CO_2 at 37 °C. All cell lines were routinely subcultured twice a week and were negative for mycoplasma as determined by the Mycotect method (Gibco).

For ELISA assays, cells (U1242, obtained from Joseph schlessinger, NYU) were grown to 80-90% confluency in growth medium (MEM with 10% FBS, NEAA, 1 mM NaPyr and 2 mM GLN) and seeded in 96-well tissue culture plates in 0.5% serum at 25,000 to 30,000 cells per well. After overnight incubation in 0.5% serum-containing medium cells were changed to serum-free medium and treated with test compound for 2 hr in a 5% CO_2, 37 °C incubator. Cells were then stimulated with ligand for 5-10 minute followed by lysis with HNTG (20 mM Hepes, 150 mM NaCl, 10% glycerol, 5 mM EDTA, 5 mM Na_3VO_4, 0.2% Triton X-100, and 2 mM NaPyr). Cell lysates (0.5 mg/well in PBS) were transferred to ELISA plates previously coated with receptor-specific antibody and which had been blocked with 5% milk in TBST (50 mM Tris-HCl ph 7.2, 150 mM NaCl and 0.1% Triton X-100) at room temperature for 30 min. Lysates were incubated with shaking for 1 hour at room temperature. The plates were washed with TBST four times

and then incubated with polyclonal anti-phosphotyrosine antibody at room temperature for 30 minutes. Excess anti-phosphotyrosine antibody was removed by rinsing the plate with TBST four times. Goat anti-rabbit IgG antibody was added to the ELISA plate for 30 min at room temperature followed by rinsing with TBST four more times. ABTS (100 mM citric acid, 250 mM Na_2HPO_4 and 0.5 mg/mL 2,2'-azino-bis(3-ethybenzthiazoline-6-sulfonic acid)) plus H_2O_2 (1.2 mL 30% H_2O_2 to 10 mL ABTS) was added to the ELISA plates to start color development. Absorbance at 410 nm with a reference wavelength of 630 nm was recorded about 15 to 30 min after ABTS addition.

The IC_{50} value measured in the PDGF receptor phosphorylation assay for the compound of formula XXIV (Example 6) was found to be 49.29 μM.

EXAMPLE 9: ASSAY MEASURING THE EGF RECEPTOR-HER2 KINASE ACTIVITY

HER2 kinase activity in whole EGFR-NIH3T3 cells was measured as described below.

Materials and Reagents

The following materials and reagents were used to conduct the assay:

a. EGF: stock concentration= 16.5 ILM; EGF 201, TOYOBO, Co., Ltd. Japan.

b. 05-101 (UBI) (a monoclonal antibody recognizing an EGFR extracellular domain).

c. Anti-phoshotyrosine antibody (anti-Ptyr) (polyclonal)(*see*, Fendley, *et al., supra*).

d. Detection antibody: Goat anti-rabbit lgG horse radish peroxidase conjugate, TAGO, Inc., Burlingame, CA.

e. TEST buffer:

Tris-HCl, pH 7.2	50 mM
NaCl	150 mM
Triton X-100	0.1

f. HNTG 5X stock:

HEPES	0.1 M

NaCl	0.75 M
Glycerol	50%
Triton X-100	1.0%

g. ABTS stock:

Citric Acid	100 mM
Na_2HPO_4	250 mM
HCl, conc.	0. 5 pM
ABTS*	0.5mg/mL

* (2,2' -azinobis(3-ethylbenzthiazolinesulfonic acid)). Keep solution in dark at 4 °C until use.

h. Stock reagents of:

EDTA 100 mM pH 7.0
Na_3VO_4 0.5 M
$Na_4(P_2O_7)$ 0.2 M

Procedure

The following protocol was used:

A. Pre-coat ELISA Plate

1. Coat ELISA plates (Corning, 96 well, Cat. #25805-96) with 05-101 antibody at 0.5 g per well in PBS, 100 µL final volume/well, and store overnight at 4 °C. Coated plates are good for up to 10 days when stored at 4 °C.

2. on day of use, remove coating buffer and replace with 100 µL blocking buffer (5% Carnation Instant Non-Fat Dry Milk in PBS). Incubate the plate, shaking, at room temperature (about 23 °C to 25 °C) for 30 minutes. Just prior to use, remove blocking buffer and wash plate 4 times with TEST buffer.

B. Seeding Cells

1. An NIH3T3 cell line overexpressing a chimeric receptor containing the EGFR extracellular domain and intracellular HER2 kinase domain can be used for this assay.

2. Choose dishes having 80-90% confluence for the experiment. Trypsinize cells and stop reaction by adding 10% fetal bovine serum. Suspend cells in DMEM medium (10% CS DMEM medium) and centrifuge once at 1500 rpm at room temperature

for 5 minutes.

3. Resuspend cells in seeding medium (DMEM, 0.5% bovine serum), and count the cells using trypan blue. Viability above 90% is acceptable. Seed cells in DMEM medium (0.5% bovine serum) at a density of 10,000 cells per well, 100 µL per well, in a 96 well microtiter plate. Incubate seeded cells in 5% CO_2 at 37 °C for about 40 hours.

C. Assay Procedures

1. Check seeded cells for contamination using an inverted microscope. Dilute drug stock (10 mg/mL in DMSO) 1:10 in DMEM medium, then transfer 5 l to a TBST well for a final drug dilution of 1:200 and a final DMSO concentration of 1%. Control wells receive DMSO alone. Incubate in 5% CO_2 at 37 °C for two hours.

2. Prepare EGF ligand: dilute stock EGF in DMEM so that upon transfer of 10 41 dilute EC37 :12 dilution), 100 nM final concentration is attained.

3. Prepare fresh HNTG sufficient for 100 µL per well; and place on ice.

HNTG (10 mL):

HNTG stock	2.0 mL
milli-Q H_2O	7.3 mL
EDTA, 100 mM, pH 7.0	0.5 mL
Na_3VO_4, 0.5 M	0.1 mL
$Na_4(P_2O_7)$, 0.2 M	0.1 mL

4. After 120 minutes incubation with drug, add prepared SGF ligand to cells, 10 µL per well, to a final concentration of 100 nM. Control wells receive DMEM alone. Incubate, shaking, at room temperature, for 5 minutes.

5. Remove drug, EGF, and DMEM. Wash cells twice with PBS. Transfer HNTG* to cells, 100 µL per well. Place on ice for 5 minutes. Meanwhile, remove blocking buffer from other ELISA plate and wash with TBST as described above.

6. With a pipette tip securely fitted to a micropipettor, scrape cells from plate and homogenize cell material by repeatedly aspirating and dispensing the HNTG* lysis buffer. Transfer lysate to a coated, blocked, and washed ELISA olate. Incubate shaking at room temperature for one hour.

7. Remove lysate and wash 4 times with TBST. Transfer freshly diluted anti-Ptyr antibody to ELISA plate at 100 µL per well. Incubate shaking at room temperature for 30 minutes in the presence of the anti-Ptyr antiserum (1:3000 dilution in TBST).

8. Remove the anti-Ptyr antibody and wash 4 times with TBST. Transfer the freshly diluted TAGO anti-rabbit IgG antibody to the ELISA plate at 100 µL per well. Incubate shaking at room temperature for 30 minutes (anti-rabbit IgG antibody: 1:3000 dilution in TBST).

9. Remove TAGO detection antibody and wash 4 times with TBST. Transfer freshly prepared ABTS/H_2O_2 solution to ELISA plate, 100 µL per well. Incubate shaking at room temperature for 20 minutes. (ABTS/H_2O_2 solution: 1.0 µL 30% H_2O_2 in 10 mL ABTS stock).

10. Stop reaction by adding 50 µL 5N H_2SO_4 (optional), and determine O.D. at 410 nm.

11. The maximum phosphotyrosine signal is determined by subtracting the value of the negative controls from the positive controls. The percent inhibitation of phosphotyrosine content for extract-containing wells is then calculated, after subtraction of the negative controls.

The IC_{50} value measured in the EGF receptor-HER2 phosphorylation assay for the compound of formula XXIV (Example 6) was found to be 8.72 µM.

EXAMPLE 10: ASSAY MEASURING THE INSULIN RECEPTOR KINASE ACTIVITY

The following protocol was used to determine whether the compounds of the present invention possessed insulin receptor tyrosine kinase activity.

Materials And Reagents

The following materials and reagents were used to measure phophotyrosine levels on the insulin receptor (indicating insulin receptor tyrosine kinase activity):

1. The preferred cell line was an NIH3T3 cell line (ATCC No. 1658) which overexpresses Insulin Receptor (H25 cells);

2. H25 cells are grown in an incubator with 5% $C0_2$ at 37 °C. The growth media is DMEM + 10% FBS (heat inactivated) + 2mm L-Glutamine;

3. For ELISA plate coating, the monoclonal anti-IR antibody named BBE is purified and used;

4. D-PBS, comprising:

KH_2P0_4	0.20 g/L (GIBCO, 310-4190AJ)
K_2HP0_4	2.16 g/L
KCl	0.20 g/L
NaCl	8.00 g/L (pH 7.2);

5. Blocking Buffer: TBST plus 5% Milk (Carnation Instant Non-Fat Dry Milk);

6. TBST buffer, comprising:

Tris-HCl	50 mM
NaCl	150 mM pH 7.2 (HCl, 1 N)
Triton X-100	0.1%

Note: Stock solution of TBS (10X) is prepared, and Triton X-100 is added to the buffer during dilution;

7. HNTG buffer, comprising:

HEPES	20mM
NaCl	150mM pH 7.2 (HCl, 1 N)
Glycerol	10%
Triton X-100	0.2%

Note: Stock solution (5X) is prepared and kept at 4 °C;

8. EDTA.HCl: 0.5 M pH 7.0 (NaOH) as 100X stock;

9. Na_3V0_4: 0.5 M as 100X stock and aliquots are kept in -80 °C;

10. $Na_4P_20_7$: 0.2 M as 100X stock;

11. Insulin from GIBCO BRL (Cat# 18125039);

12. Polyclonal antiserum Anti-phosphotyrosine: rabbit sera or UB40 monoclonal antibody specific for phosphotyrosine.

13. Detection antibody, preferably goat anti-rabbit IgG, POD conjugate, Tago (Cat. No. 4520: Lot No. 1802): Tago, Inc., Burlingame, CA;

14. ABTS solution, comprising:

Citric acid	100 mM
Na_2HP0_4	250 mM pH 4.0 (1 N HCl)
ABTS	0.5 mg/mL

wherein ABTS is 2,2'-azinobis (3-ethylbenathiazoline sulfonic acid) and stored in the dark at 4 °C and discarded when it turns green;

15. Hydrogen Peroxide: 30% solution is kept in the dark and at 40 °C.

Protocol

All the following steps are conducted at room temperature unless it is specifically indicated. All ELISA plate washings are performed by rinsing the plate with tap water three times, followed by one TBST rinse. All plates were tapped dry with paper towels prior to use.

A. Cell Seeding:

1. The cells were grown in tissue culture dish (10 cm, Corning 25020-100) to 80-90% confluence and harvested with Trypsin-EDTA (0.25%, 0.5 mL/D-100, GIBCO);

2. Resuspend the cells in fresh DMEM + 10% FBS + 2mM L-Glutamine, and transfer to 96 - well tissue culture plate (Corning, 25806-96) at 20,000 cells/well (100 µL/well). The cells are then incubated for 1 day. Following such incubation, 0.01% serum medium (90/µL) replaces the old media and the cells incubate in 5% $C0_2$ and 37 °C overnight.

B. ELISA Plate Coating and Blocking:

1. Coat the ELISA plate (Corning 25805-96) with Anti-IR Antibody at 0.5 µg/well in 100 µL PBS at least 2 hours.

2. Remove the coating solution, and replace with 100 u; blocking Buffer, and shake for 30 minutes. Remove the blocking buffer and wash the plate just before adding lysate.

C. Assay Procedures

1. The drugs are tested in serum-free condition.

2. Dilute drug stock (in 100% DMSO) 1:10 with DMEM in 96-well poly-

propylene plate, and transfer 10 µL/well of this solution to the cells to achieve final drug dilution 1:100, and final DMSO concentration of 1.0%. Incubate the cells in 5% $C0_2$ at 37 °C for 2 hours.

3. Prepare fresh cells lysis buffer (HNTG*)

HNTG (5x)	2 mL
EDTA	0.1 mL
Na_3V0_4	0.1 mL
$Na_4P_20_7$	0.1 mL
H_20	7.3 mL
HNTG*	10 mL

4. After drug incubation for two hours, transfer 10 µL/well of 1µM insulin in PBS to the cells (Final concentration = 100 nM), and incubate at 5% $C0_2$ at 37 °C for 10 minutes.

5. Remove media and add 100 µL/well HNTG* and shake for 10 minutes. Look at cells under microscope to see if they are adequately lysed.

6. Using a 12-channel pipette, scrape the cells from the plate, and homogenize the lysate by repeat aspiration and dispense. Transfer all the lysate to the antibody coated ELISA plate, and shake for 1 hour.

7. Remove the lysate, wash the plate, transfer anti-pTyr (1:3,000 with TBST) 100 µL/well, and shake for 30 minutes.

8. Remove anti-pTyr, wash the plate, transfer Tago (1:3,000 with TBST) 100 µL/well, and shake for 30 minutes.

9. Remove detection antibody, wash the plate, and transfer fresh ABTS/H_20_2 (1.2 µL H_20_2 to 10 mL ABTS) 100 µL/well to the plate to start color development.

10. Measure OD in Dynatec MR5000, which is connected to Ingres. All following steps should follow Ingres instruction.

The IC_{50} value measured in the insulin receptor phosphorylation assay for the compound of formula XXIV (Example 6) was found to be 21.94 µM.

EXAMPLE 11: ASSAY MEASURING THE FLK-1 RECEPTOR KINASE ACTIVITY

An ELISA assay was conducted to measure the kinase activity of the FLK-1 receptor and more specifically, the inhibition or activation of TK activity on the FLK-1 receptor. Specifically, the following assay was conducted to measure kinase activity of the FLK-1 receptor in cells genetically engineered to express FLK-1.

Materials And Methods

The following reagents and supplies were used:

a. Corning 96-well ELISA planes (Corning Catalog No. 25805-96);

b. Cappel goat anti-rabbit IgG (catalog no. 55641);

c. PBS (Gibco Catalog No. 450-1300EB);

d. TBSW Buffer (50 mM Tris pH 7.2), 150 mM NaCl and 0.1% Tween-20);

e. Ethanolamine stock (10% ethanolamine (pH 7.0), stored at 4 °C);

f. HNTG buffer (2O mM HEPES buffer (pH 7.5), 150 mM NaCl, 0.2% Triton X-100, and glycerol);

g. EDTA (0.5 M (pH 7.0) as a 100X stock);

h. Sodium ortho vanadate (0.5 M as a 100X stock);

i. Sodium pyro phosphate (0.2M as a 100X stock);

j. NUNC 96 well V bottom polypropylene plates (Applied Scientific Catalog No. AS-72092);

k. NIH3T3 C7#3 Cells (FLK-1 expressing cells);

l. DMEM with 1X high glucose L Glutamine (catalog No. 11965-050);

m. FBS, Gibco (catalog no. 16000-028);

n. L-glutamine, Gibco (catalog no. 25030-016);

o. VEGF, PeproTech, Inc. (catalog no. 100-20)(kept as 1 μg/100 μL stock in Milli-Q dH_2O and stored at -20 °C;

p. Affinity purified anti-FLK-1 antiserum;

q. UB40 monoclonal antibody specific for phosphotyrosine (see, Fendley, *et al.*, 1990, *Cancer Research* 50:1550-1558);

r. EIA grade Goat anti-mouse IgG-POD (BioRad catalog no. 172-1011);

s. 2,2-azino-bis(3-ethylbenz-thiazoline-6-sulfonic acid (ABTS) solution (100mM citric acid (anhydrous), 250 mM Na_2HPO_4 (pH 4.0), 0.5 mg/mL ABTS (Sigma catalog no. A-1888)), solution should be stored in dark at 4 °C until ready for use;

t. H_2O_2 (30% solution)(Fisher catalog no. H325);

u. ABTS/H_2O_2 (15 mL ABTS solution, 2 µL H_2O_2) prepared 5 minutes before use and left at room temperature;

v. 0.2 M HCl stock in H_2O;

w. dimethylsulfoxide (100%)(Sigma Catalog No. D-8418); and

y. Trypsin-EDTA (Gibco BRL Catalog No. 25200-049).

Protocol

The following protocol was used for conducting the assay:

1. Coat Corning 96-well elisa plates with 1.0 µg per well Cappel Anti-rabbit IgG antibody in 0.1 M Na_2CO_3 pH 9.6. Bring final volume to 150 µL per well. Coat plates overnight at 4 °C. Plates can be kept up to two weeks when stored at 4 °C.

2. Grow cells in Growth media (DMEM, supplemental with 2.0mM L-Glutamine, 10% FBS) in suitable culture dishes until confluent at 37 °C, 5% CO_2.

3. Harvest cells by trypsinization and seed in Corning 25850 polystyrene 96-well roundbottom cell plates, 25.000 cells/well in 200 µL of growth media.

4. Grow cells at least one day at 37 °C, 5% CO_2.

5. Wash cells with D-PBS 1X.

6. Add 200 µL/well of starvation media (DMEM, 2.0mM 1-Glutamine, 0.1% FBS). Incubate overnight at 37 °C, 5% CO_2.

7. Dilute Compounds 1:20 in polypropylene 96 well plates using starvation media. Dilute dimethylsulfoxide 1:20 for use in control wells.

8. Remove starvation media from 96 well cell culture plates and add 162 µL of fresh starvation media to each well.

9. Add 18 µL of 1:20 diluted Compound dilution (from step 7) to each well

plus the 1:20 dimethylsulfoxide dilution to the control wells (+/- VEGF), for a final dilution of 1:200 after cell stimulation. Final dimethylsulfoxide is 0.5%. Incubate the plate at 37 °C, 5% CO_2 for two hours.

10. Remove unbound antibody from ELISA plates by inverting plate to remove liquid. Wash 3 times with TBSW + 0.5% ethanolamine, pH 7.0. Pat the plate on a paper towel to remove excess liquid and bubbles.

11. Block plates with TBSW + 0.5% Ethanolamine, pH 7.0, 150 µL per well. Incubate plate thirty minutes while shaking on a microliter plate shaker.

12. Wash plate 3 times as described in step 10.

13. Add 0.5 µg/well affinity purified anti-FLU-1 polyclonal rabbit antiserum. Bring final volume to 150 µL/well with TBSW + 0.5% ethanolamine pH 7.0. Incubate plate for thirty minutes while shaking.

14. Add 180 µL starvation medium to the cells and stimulate cells with 20 µL/well 10.0mM sodium ortho vanadate and 500 ng/mL VEGF (resulting in a final concentration of 1.0 mM sodium ortho vanadate and 50 ng/mL VEGF per well) for eight minutes at 37 °C, 5% CO_2. Negative control wells receive only starvation medium.

15. After eight minutes, media should be removed from the cells and washed one time with 200 µL/well PBS.

16. Lyse cells in 150 µL/well HNTG while shaking at room temperature for five minutes. HNTG formulation includes sodium ortho vanadate, sodium pyro phosphate and EDTA.

17. Wash ELISA plate three times as described in step 10.

18. Transfer cell lysates from the cell plate to ELISA plate and incubate while shaking for two hours. To transfer cell lysate pipette up and down while scrapping the wells.

19. Wash plate three times as described in step 10.

20. Incubate ELISA plate with 0.02 µg/well UB40 in TBSW + 05% ethanolamine. Bring final volume to 150 µL/well. Incubate while shaking for 30 minutes.

21. Wash plate three times as described in step 10.

22. Incubate ELISA plate with 1:10,000 diluted EIA grade goat anti-mouse IgG conjugated horseradish peroxidase in TBSW + 0.5% ethanolamine, pH 7.0. Bring final volume to 150 µL/well. Incubate while shaking for thirty minutes.

23. Wash plate as described in step 10.

24. Add 100 µL of ABTS/H_2O_2 solution to well. Incubate ten minutes while shaking.

25. Add 100 µL of 0.2 M HCl for 0.1 M HCl final to stop the color development reaction. Shake 1 minute at room temperature. Remove bubbles with slow stream of air and read the ELISA plate in an ELISA plate reader at 410 nm.

The IC_{50} value measured in the FLK-1 receptor phosphorylation assay for the compound of formula XXIV (Example 6) was found to be 4.84 µM.

EXAMPLE 12: ASSAY MEASURING THE EFFECT OF INDOLINONE COMPOUNDS ON THE GROWTH OF A431 CELLS

The following assay measures growth rates for A431 cells.

Materials

96-well flat bottom sterile plates
96-well round bottom sterile plates
sterile 25 mL or 100 mL reservoir
pipets, multi-channel pipetman
sterile pipet tips
sterile 15 mL and 50 mL tubes

Reagents

0.4% SRB in 1% acetic acid
10 mM Tris base
10% TCA
1% acetic acid
sterile DMSO (Sigma)
compound in DMSO (100 mM or less stock solution)
Trypsin-EDTA (GIBCO BRL)

Cell line:

A431 cells (ATCC CRL 1555)

Growth medium:

2% calf serum/DMEM + 2 mM glutamine, Pen/Strep

Protocol:

Day 0: Cell Plating:

This part of assay is carried out in a laminar flow hood.

1. Trypsinize cells. Transfer 200 µL of cell suspension to 10 mL of isotone. Count cells with a Coulter Counter.

2. Dilute cells in growth medium to 60,000 cell/mL. Transfer 100 µL of cells to each well in a 96-well flat bottom plate to give 6000 cells/well.

3. Use half of plate (4 rows) for each comound and quadruplicate wells for each comound concentration, and a set of 4 wells for medium control.

4. Gently shake plates to allow for uniform attachment of the cells.

5. Incubate the plates at 37 °C in a 10% CO_2 incubator.

Day 1: Addition of Compound:

This part of assay is carried out in a laminar flow hood.

1. In a 96-well round bottom plate, add 120 µL of growth medium containing 2X final % DMSO found in highest screening concentration of compound to columns 1 to 11. For example, if the highest concentration is 100 µL, and this is made from a 100 mM stock, 1X DMSO is 0.1%, so 2X DMSO is 0.2%. This plate is used to titrate out the compound, 4 rows per compound.

2. In a sterile 15 mL tube, make a 2X solution of the highest screening concentration of compound in growth medium plus 2X DMSO. 1 mL per cell line is needed. The starting concentration of the compound is usually 100 µM but this concentration may vary depending upon the solubility of the compound.

3. Transfer 240 µL of the 2X starting compound solution to qudruplicate

wells in column 12 of the 96-well round bottom plate. Do 1:2 serial dilutions across the plate from right to left by transferring 12 µL from column 12 to column 11, column 11 to 10 and so on through column 2. Transfer 100 µL of compound dilutions, and 100 µL of medium in column 1, onto 100 µL medium on cells in corresponding wells of 96-well flat bottom plate. Total volume per well should be 200 µL.

4. Return the plate to the incubattor and incubate for 3 days.

Day 4: Development of Assay

This party of assay is carried out on the bench.

1. Aspirate or pour off medium. Add 200 µL cold 10% TCA to each well to fix cells. Incubate plate for at least 60 min. at 4 °C.

2. Discard TCA and rinse wells 5 times with tap water. Dry plates upside down on paper towels.

3. Stain cells with 100 µL/well 0.4% SRB for 10 min.

4. Pour of SRB and rinse wells 5 times with 1% acetic acid. Dry plates completely upside down on paper towels.

5. Solubilize dye with 100 µL/well 10 mM Tris base for 5-10 min. on shaker.

6. Read plates on Dynatech ELISA Plate REader at 570 nm with reference at 630 nm.

Compound of formula XXIV (Example 6) inhibited the growth rate of A431 cells and the IC_{50} was found to be 0.712 µM.

EXAMPLE 13: ASSAY MEASURING THE EFFECT OF INDOLINONE COMPOUNDS ON THE GROWTH OF SKOV3 CELLS

The assay is the same as the assay set forth in EXAMPLE 10, except that SKOV3 cells (ATCC HTB77) are used instead of A431 cells.

Compound of formula XXIV (Example 6) inhibited the growth rate of SKOV3 cells and the IC_{50} was found to be 1.08 µM.

EXAMPLE 14: ASSAY MEASURING THE EFFECT OF INDOLINONE COMPOUNDS ON THE GROWTH OF C6 CELLS

The assay is the same as the assay set forth in EXAMPLE 10, except that C6 cells (ATCC 107) are used instead of A431 cells.

Compound of formula XXIV (Example 6) inhibited the growth rate of C6 cells and the IC_{50} was found to be 1.55 μM.

EXAMPLE 15: PDGF-, EGF-, AND FGF-INDUCED BRDU INCORPORATION ASSAY

Materials and Reagents

(1) PDGF: human PDGF B/B; 1276-956, Boehringer Mannheim, Germany

(2) BrdU Labeling Reagent: 10 mM, in PBS (pH7.4), Cat. No. 1 647 229, Boehringer Mannheim, Germany.

(3) FixDenat: fixation solution (ready to use), Cat. No. 1 647 229, Boehringer Mannheim, Germany.

(4) Anti-BrdU-POD: mouse monoclonal antibody conjugated with peroxidase, Cat. No. 1 647 229, Boehringer Mannheim, Germany.

(5) TMB Substrate Solution: tetramethylbenzidine (TME), ready to use, Cat. No. 1 647 229, Boehringer Mannheim, Germany.

(6) PBS Washing Solution: 1X PBS, pH 7.4, made in house.

(7) Albumin, Bovine (BSA): fraction V powder; A- 8551, Sigma Chemical Co., USA.

(8) 3T3 cell line genetically engineered to express human PDGF-R.

Protocol

(1) Cells are seeded at 8000 cells/well in DMEM, 10% CS, 2 mM Gln in a 96 well plate. Cells are incubated overnight at 37 °C in 5% CO_2.

(2) After 24 hours, the cells are washed with PBS, and then are serum starved serum free medium (0% CS DMEM with 0.1% BSA) for 24 hours.

(3) On day 3, ligand (PDGF=3.8 nM, prepared in DMEM with 0.1% BSA) and test compounds are added to the cells simultaneously. The negative control wells

receive serum free DMEM with 0.1% BSA only; the positive control cells receive the ligand (PDGF) but no test compound. Test compounds are prepared in serum free DMEM with ligand in a 96 well plate, and serially diluted for 7 test concentrations.

(4) After 20 hours of ligand activation, diluted BrdU labeling reagent (1:100 in DMEM, 0.1% BSA) is added and the cells are incubated with BrdU (final concentration=10 μM) for 1.5 hours.

(5) After incubation with labeling reagent, the medium is removed by decanting and tapping the inverted plate on a paper towel. FixDenat solution is added (50 μL/well) and the plates are incubated at room temperature for 45 minutes on a plate shaker.

(6) The FixDenat solution is thoroughly removed by decanting and tapping the inverted plate on a paper towel. Milk is added (5% dehydrated milk in PBS, 200 μL/well) as a blocking solution and the plate is incubated for 30 minutes at room temperature on a plate shaker.

(7) The blocking solution is removed by decanting and the wells are washed once with PBS. Anti-BrdU-POD solution (1:100 dilution in PBS, 1% BSA) is added (100 μL/well), and the plate is incubated for 90 minutes at room temperature on a plate shaker.

(8) The antibody conjugate is thoroughly removed by decanting and rinsing the wells 5 times with PBS, and the space is dried by inverting and tapping on a paper towel.

(9) TMB substrate solution is added (100 μL/well) and incubated for 20 minutes at room temperature on a plate shaker until color development is sufficient for photometric detection.

(10) The absorbance of the samples are measured at 410 nm (in "dual wavelength" mode with a filter reading at 490 nm, as a reference wavelength) on a Dynatech ELISA plate reader.

The IC_{50} values were measured for several of the compounds of the invention. These values are depicted in Table 12.

TABLE 12

Compound	EGF-Induced BrdU Incorp. IC_{50} (μM)	PDGF-Induced BrdU Incorp. IC_{50} (μM)	FGF-Induced BrdU Incorp. IC_{50} (μM)
AP-001	15.6	< 3.1	5.2
AP-002	10.2	9.6	17.3
AP-003	> 100	> 100	> 100
AP-004	26	30	20.8
AP-005	21.2	17.2	9.5
AP-006	69.6	15.7	22.5
AP-007	50.1	20.1	21.6
AP-008	53.4	6.4	7.8
AP-009	46.8	> 100	> 100
AP-010	> 100	25.8	24.2
AP-011	22.5	20	27.2
AP-012	28.2	29.7	29
AP-013	> 100	> 100	> 100
AP-014	50.1	48.4	36.3
AP-015	53.4	64.3	24.1
AP-016	46.8	57.5	22.9
AP-017	> 100	> 100	> 100
AP-018	N/A*	40	41.8
AP-019	> 50	> 50	31.4
AP-021	4.4	4.8	2.4
AP-022	> 50	> 50	> 50
AP-023	> 50	> 50	> 50
AP-024	> 50	> 50	> 50
AP-025	> 50	> 50	> 50
AP-026	43	> 50	28
AP-027	> 50	> 50	19.2
AP-028	> 50	> 50	> 50
AP-029	> 50	> 50	44.4
AP-030	7.1	8.7	3.8
AP-031	6.3	8.6	2.5
AP-032	9.8	> 50	> 50
AP-033	9.8	12.4	6.5

*: Biological data is not available for this compound.

The IC_{50} values were also measured for the compound of formula XXIV (Example 6) are depicted in Table 13:

TABLE 13

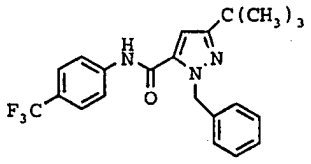
Assay	IC_{50} (μM)
3T3EGFR EGF	7.03
3T3EGFR PDGF	7.00

EXAMPLE 16: *IN VIVO* TUMOR INHIBITION

In vivo tumor inhibition was measured using a subcutaneous Xenograft model. Mice (BALB/c, nu/nu) were implanted with Calu-6 human lung carcinoma cells and the ability of compounds of the invention to inhibit tumor growth were measured.

Calu-6 cells were maintained in Ham's F10 supplemented with 10% fetal bovine serum (FBS) and 2 mM glutamine (GLN). Cells were harvested at or near confluence with 0.05% Trypsin-EDTA and pelleted at 450 x g for 10 min. Pellets were resuspended in sterile PBS or media (without FBS) to a particular concentration and the cells were implanted into the hindflank of mice. Tumor growth was measured over 3 to 6 weeks using venier calipers. Tumor volumes were calculated as a product of length x width x height unless otherwise indicated. AP-001 was solubilized in 50 - 100 μL vehicle (VPD:D5W). The compounds were delivered by IP injection at different doses.

Table 14, below, illustrates the ability of compound AP-001 to inhibit tumor growth.



(AP-001)

Increased efficacy can be obtained by optimizing dosing regiments. For example, the amount and timing of the doses can be varied and tested using procedures known in the art and described herein.

As Table 14 indicates, at 25 mg/kg/day, AP-001 significantly inhibited up to 70% the growth of Calu-6 cells in a subcutaneous xenograft model. No mortality was seen at that dose. No inhibition was seen with 50 and 75 mg/kg/day, but the compound formed a gel upon dissolution in VPD:D5W.

Table 14

Dosage mg/kg/day	Day	Average Tumor Volume (mm^3)	% Inhibition
25	8	78.90±12.43	58
	13	90.95±13.84	61
	15	99.89±21.82	70
	19	199.35±47.64	64
50	8	135.05±21.89	29
	13	210.18±39.94	9
	15	235.53±39.33	29
	19	488.07±116.59	11
75	8	80.39±16.51	58
	13	203.87±35.70	12
	15	270.28±61.49	18
	19	432.78±56.08	21
Control*	8	190.09±17.32	
	13	230.61±23.39	
	15	331.39±34.92	
	19	548.06±47.55	

*: By definition, control samples show no inhibition.

EXAMPLE 17: FLK-1 AUTOPHOSPHORYLATION ASSAY *IN VITRO*

The following protocol describes the ELISA procedures used to determine the FLK-1 autophosphorylation activity *in vitro*. The procedure also describes the protocol for the initial screening of indolinone compounds.

Reagents and Supplies

1. 15 cm tissue culture dishes.

2. FLK-1/NIH cells: NIH fibroblast line overexpressing human FLK-1 clone 3 (MPI,

Martinsried, Germany).

3. Growth medium: DMEM plus heat-inactivated 10% FBS and 2 mM Glutamine (Gibco-BRL, Gaithersburg, USA).

4. Starvation medium: DMEM plus 0.5% heat-inactivated FBS and 2 mM Glutamine (Gibco-BRL, Gaithersburg, USA).

5. Corning 96-well ELISA plates (Corning Cat. # 25805-96).

6. L4 or E38: monoclonal antibody specific for FLK-1; purified by protein-A agarose affinity chromatography (SUGEN, Inc., Redwood City, CA).

7. PBS (Dulbecco's Phosphate-Buffered Saline) (Gibco Catalog # 450-1300EB)

KCL	2.7 mM
KH_2PO_4	1.1 mM
$MgCl_2$ (anhydrous)	0.5 mM
NaCl	138 mM
Na_2HPO_4	8.1 mM

8. HNTG

HEPES/HCl	20 mM
NaCl	150 mM
Glycerol	10%
Triton X-100	1.0%
PMSF	1 mM (optional)

9. Pierce BCA protein determination kit.

10. Blocking buffer

5% Carnation instant milk in PBS

11. TBST, pH 7.0

Tris/HCl	50 mM
NaCl	150 mM

Triton X-100	0.1%

12. Kinase buffer

Tris/HCl	25 mM
NaCl	100 mM
$MnCl_2$	10 mM
Glycerol	2%

Add the following just prior to use:

DTT	0.5 mM
Triton X-100	0.1%

13. Kinase stop solution

EDTA	200 mM

14. Biotinylated 4G10, specific for phosphotyrosine (UBI Cat # 16-103, Lake Placid, NY).

15. AB kit (Vector Laboratories, Cat # PK 4000, Burlingame, CA).

16. DMSO

17. NUNC 96-well V bottom polypropylene plates for compounds (Applied Scientific, Cat # AS-72092).

18. ABTS solution

Citric Acid (anhydrous)	100 mM
Na_2HPO_4, pH 4.0	250 mM
ABTS	0.5 mg/mL

19. Hydrogen perosice 30% solution (Fisher Cat # H325). Store in the dark at 4 °C until ready to use.

20. $ABTS/H_2O_2$

ABTS solution	15 mL
H_2O_2	2 µL

21. ATP (Sigma Cat # A-7699).

22. TBST, pH 7.0

Tris/HCl	50 mM
NaCl	150 mM

23. 20% DMSO in TBS pH 7.0.

Procedure

A. Cell Growth and Lysate Preparation

1. Seed cell into growth medium and grow for 2-3 days foo 90-100% confluency at 37 °C and 5% CO_2. Do not exceed passage #20.

2. Remove the medium and wash the cells twice with PBS. Lyse with HNTG lysis buffer. Collect all lysates and mix them on a vortex for 20-30 seconds.

3. Remove insolubel material by centrifugation (5-10 min at ~10,000xg).

4. Determine the protein concentration via BCA kit.

5. Aliquot lysates into 1 mg aliquots, store at -80 °C.

B. Assay Procedure

1. Coat Corning 96-well ELISA plates with 2 ug/well of purified L4 (or E38) in 100 µL of PBS overnight at 4 °C.

2. Remove unbound proteins from wells by inverting the plate to remove the liquid. Wash one time with d-H_2O, pat plate on paper towel to remove excess liquid.

3. Block plates with 150 µL blocking buffer per well. Incubate for 45-60 minutes while shaking at 4 °C.

4. Remove the blocking buffer and wash the ELISA plate three times with d-H_2O and one time with TBST. Pat plate on paper towel to remove excess liquid.

5. Dilute lysate in PBS to give final concentration of 50 ug/100 µL. Add 100 µL of diluted lysate per well. Incubate with shaking at 4 °C overnight.

6. Remove unbound proteins from wells by inverting the plate. Wash as in step 4.

7. Add 80 µL of kinase buffer to the wells (negative control wells get 90 µL).

8. Dilue compounds (normally 10 fold) into wells of a polypropylene plate containing 20% DMSO in TBS.

9. Add 10 µL of the prediluted compounds to the ELISA wells containing immobilized FLK-1 and shake. Control wells receive no compound.

10. From stock 1mM ATP prepare a 0.3 mM ATP solution in d-H_2O (kinase buffer may also be used).

11. Add 10 µL of 0.3 mM ATP to all wells except the negative controls. Incubate for 60 min at room temperature with shaking.

12. After 10-15 min stop the kinase reaction with the addition of 11 µL 200 mM EDTA. Let shake for 1-2 min.

13. Wash the ELISA plate 4 times with d-H_2O and twice with TBST.

14. Add 100 µL of 1:5000 fold diluted biotinylated 4G10 (in TBST) to all wells. Incubate 45 min with shaking at room temperature.

15. While the above is incubating, add 50 µL of solutions A & B (of the ABC kit)/10 mL of TBST. These must be combined ~30 min prior to use.

16. Wash plates as in step 4.

17. Add 100 µL of the above preformed complex (solution A & B in TBST) to all wells. Incubate 30 min with shaking at room temperature.

18. Wash plates as in step 4.

19. Add 100 µL ABTS/H_2O_2 solution. Shake at room temperature of 5-10 min.

20. When the color in the positive control wells reaches an absorbance of about 0.35-0.4, read plates on Dynatech MR7000 ELISA reader.

Test filter: 630 nm

Reference filter: 410 nm

The IC_{50} value measured in the above assay for the compound of formula XXIV (Example 6) was found to be > 50 µM.

EXAMPLE 18: HUV-EC-C ASSAY

The following protocol may also be used to measure a compound's activity against PDGF-R, FGF-R or Flk-l/KDR, all of which are naturally expressed by HUV-EC cells.

DAY 0

1. Wash and trypsinize HUV-EC-C cells (human umbilical vein endothelial cells, (American Type Culture Collection; catalogue no. 1730 CRL). Wash with Dulbecco's phosphate-buffered saline (D-PBS; obtained from Gibco BRL, catalogue no. 14190-029) 2 times at about 1 mL/10 CM^2 of tissue culture flask. Trypsinize with 0.05% trypsin-EDTA in non-enzymatic cell dissociation solution (Sigma Chemical Company; catalogue no. C-1544). The 0.05% trypsin was made by diluting 0.25% trypsin/1 mM EDTA (Gibco; catalogue no. 25200-049) in the cell dissociation solution. Trypsinize with about 1 mL/25-30 cm^2 Of tissue culture flask for about 5 minutes at 37 °C. After cells have detached from the flask, add an equal volume of assay medium and transfer to a 50 mL sterile centrifuge tube (Fisher Scientific; catalogue no. 05-539-6).

2. Wash the cells with about 35 mL assay medium in the 50 mL sterile centrifuge tube by adding the assay medium, centrifuge for 10 minutes at approximately 200xg, aspirate the supernatant, and resuspend with 35 mL D-PBS. Repeat the wash two more times with D-PBS, resuspend the cells in about 1 mL assay medium/15 cm^2 of tissue culture flask. Assay medium consists of F12K medium (Gibco BRL; catalogue no. 21127-014) + 0.5% heat-inactivated fetal bovine serum. Count the cells with a Coulter Counter®v Coulter Electronics, Inc.) and add assay medium to the cells to obtain a concentration of 0.8-1.0x10^5 cells/mL.

3. Add cells to 96-well flat-bottom plates at 100 µL/well or 0.8-1.0x10^4 cells/well; incubate ~24 h at 37 C, 5% $C0_2$.

DAY 1

1. Make up two-fold drug titrations in separate 96-well plates, generally 50 µM on down to 0 µM. Use the same assay medium as mentioned in day 0, step 2 above. Titrations are made by adding 90 µL/well of drug at 200 µM (4X the final well concentration) to the top well of a particular plate column. Since the stock drug concentration is usually 20 mM in DMSO, the 200 µM drug concentration contains 2% DMSO.

Therefore, diluent made up to 2% DMSO in assay medium (Fl2K + 0.5% fetal bovine serum) is used as diluent for the drug titrations in order to dilute the drug but keep

the DMSO concentration constant. Add this diluent to the remaining wells in the column at 60 µL/well. Take 60 µL from the 120 µL of 200 µM drug dilution in the top well of the column and mix with the 60 µL in the second well of the column. Take 60 µL from this well and mix with the 60 µL in the third well of the column, and so on until two-fold titrations are completed. When the next-to-the-last well is mixed, take 60 µL of the 120 µL in this well and discard it. Leave the last well with 60 µL of DMSO/media diluent as a non-drug-containing control. Make 9 columns of titrated drug, enough for triplicate wells each for 1) VEGF (obtained from Pepro Tech Inc., catalogue no. 100-200, 2) endothelial cell growth factor (ECGF) (also known as acidic fibroblast growth factor, or aFGF) (obtained from Boehringer Mannheim Biochemica, catalogue no. 1439 600); or, 3) human PDGF B/B (1276-956, Boehringer Mannheim, Germany) and assay media control. ECGF comes as a preparation with sodium heparin.

2. Transfer 50 µL/well of the drug dilutions to the 96-well assay plates containing the 0.8-1.0x10^4 cells/100 µL/well of the HUV-EC-C cells from day 0 and incubate ~2 h at 37 °C, 5% $C0_2$.

3. In triplicate, add 50 µL/well of 80 µg/mL VEGF, 20 ng/mL ECGF, or media control to each drug condition. As with the drugs, the growth factor concentrations are 4X the desired final concentration. Use the assay media from day 0 step 2 to make the concentrations of growth factors. Incubate approximately 24 hours at 37 °C, 5% CO_2. Each well will have 50 µL drug dilution, 50 µL growth factor or media, and 100 µL cells, = 200 µL/well total. Thus the 4X concentrations of drugs and growth factors become 1X once everything has been added to the wells.

DAY 2

1. Add ^{3}H-thymidine (Amersham; catalogue no. TRK-686) at 1 µCi/well (10 µL/well Of 100 µCi/mL solution made up in RPMI media + 10% heat-inactivated fetal bovine serum) and incubate ~24 h at 37 °C, 5% $C0_2$. Note: ^{3}H-thymidine is made up in RPMI media because all of the other applications for which we use the ^{3}H-thymidine involve experiments done in RPMI. The media difference at this step is probably not significant. RPMI was obtained from Gibco BRL, catalogue no. 11875-051.

DAY 3

1. Freeze plates overnight at 20 °C.

DAY 4

1. Thaw plates and harvest with a 96-well plate harvester (Tomtec Harvester 96®) onto filter mats (Wallac; catalogue no. 1205-401); read counts on a Wallac Betaplate liquid scintillation counter.

The IC_{50} values measured for the compound of formula XXIV (Example 6) in the HUVEC vEGF assay was 0.68 μM and in the HUVEC aFGF assay was 0.63 μM. Also, Compound AV-002 showed greater inhibition of FGF-stimulation of cell proliferation than Compound AV-003 (8.0 μM versus 16.2 μM, respectively), and both were more potent than Compound AV-004 (>50 μM).

EXAMPLE 19: HER-2 ELISA

HER2 kinase activity in whole EGFR-NIH3T3 cells can be measured as described below:

Materials and Reagents.

The following materials and reagents were used to conduct the assay:

a. EGF: stock concentration: 16.5 ILM; EGF 201, TOYOBO, Co., Ltd. Japan.

b. 05-101 (UBI) (a monoclonal antibody recognizing an EGFR extracellular domain).

c. Anti-phosphotyrosine antibody (anti-Ptyr) (polyclonal)(see, Fendley, et al., supra).

d. Detection antibody: Goat anti-rabbit lgG horse radish peroxidase conjugate, TAGO, Inc., Burlingame, CA.

e. TBST buffer:

Tris-HCl, pH 7.2	50 mM
NaCl	150 mM
Triton X-100	0.1

f. HNTG 5X stock:

HEPES	0.1 M
NaCl	0.75 M
Glycerol	50%
Triton X-100	1.0%

g. ABTS stock:

Citric Acid	100 mM
Na2HPO4	250 mM
HCl, conc.	0.5 pM
ABTS*	0.5 mg/mL

* (2,2'-azinobis(3-ethylbenzthiazolinesulfonic acid)). Keep solution in dark at 4 °C until use.

h. Stock reagents of:

EDTA 100 mM pH 7.0
Na_3VO_4 0.5 M
$Na_4(P_2O_7)$ 0.2 M

Procedure

The following protocol was used:

A. Pre-coat ELISA Plate

1. Coat ELISA plates (Corning, 96 well, Cat. #25805-96) with 05-101 antibody at 0.5 g per well in PBS, 100 µL final volume/well, and store overnight at 4 °C. Coated plates are good for up to 10 days when stored at 4 °C.

2. On day of use, remove coating buffer and replace with 100 µL blocking buffer (5% Carnation Instant Non-Fat Dry Milk in PBS). Incubate the plate, shaking, at room temperature (about 23 °C to 25 °C) for 30 minutes. Just prior to use, remove blocking buffer and wash plate 4 times with TBST buffer.

B. Seeding Cells

1. An NIH3T3 cell line overexpressing a chimeric receptor containing the EGFR extracellular domain and intracellular HER2 kinase domain can be used for this assay.

2. Choose dishes having 80-90% confluence for the experiment. Trypsinize cells and stop reaction by adding 10% fetal bovine serum. Suspend cells in DMEM medium (10% CS DMEM medium) and centrifuge once at 1500 rpm, at room temperature for 5 minutes.

3. Resuspend cells in seeding medium (DMEM, 0.5% bovine serum), and count the cells using trypan blue. Viability above 90% is acceptable. Seed cells in DMEM

medium (0.5% bovine serum) at a density of 10,000 cells per well, 100 µL per well, in a 96 well microtiter plate. Incubate seeded cells in 5% CO_2 at 37 °C for about 40 hours.

C. Assay Procedures

1. Check seeded cells for contamination using an inverted microscope. Dilute drug stock (10 mg/mL in DMSO) 1:10 in DMEM medium, then transfer 5 µL to a TBST well for a final drug dilution of 1:200 and a final DMSO concentration of 1%. Control wells receive DMSO alone. Incubate in 5% CO_2 at 37 °C for two hours.

2. Prepare EGF ligand: dilute stock EGF in DMEM so that upon transfer of 10 µL dilute EGF (1:12 dilution), 100 nM final concentration is attained.

3. Prepare fresh HNTG* sufficient for 100 µL per well; and place on ice.

HNTG* (10 mL):	
HNTG stock	2.0 mL
milli-Q H_20	7.3 mL
EDTA, 100 mM, pH 7.0	0.5 mL
Na_3VO_4, 0.5 M	0.1 mL
$Na_4(P_2O_7)$, 0.2 M	0.1 mL

4. After 120 minutes incubation with drug, add prepared SGF ligand to cells, 10 µL per well, to a final concentration of 100 nM. Control wells receive DMEM alone. Incubate, shaking, at room temperature, for 5 minutes.

5. Remove drug, EGF, and DMEM. Wash cells twice with PBS. Transfer HNTG* to cells, 100 µL per well. Place on ice for 5 minutes. Meanwhile, remove blocking buffer from other ELISA plate and wash with TBST as described above.

6. With a pipette tip securely fitted to a micropipettor, scrape cells from plate and homogenize cell material by repeatedly aspirating and dispensing the HNTG* lysis buffer. Transfer lysate to a coated, blocked, and washed ELISA plate. Incubate shaking at room temperature for one hour.

7. Remove lysate and wash 4 times with TBST. Transfer freshly diluted anti-Ptyr antibody to ELISA plate at 100 µL per well. Incubate shaking at room temperature for 30 minutes in the presence of the anti-Ptyr antiserum (1:3000 dilution in TBST).

8. Remove the anti-Ptyr antibody and wash 4 times with TBST. Transfer the freshly diluted TAGO anti-rabbit IgG antibody to the ELISA plate at 100 µL per well.

Incubate shaking at room temperature for 30 minutes (anti-rabbit IgG antibody: 1:3000 dilution in TBST).

9. Remove TAGO detection antibody and wash 4 times with TBST. Transfer freshly prepared ABTS/H_2O_2 solution to ELISA plate, 100 µL per well. Incubate shaking at room temperature for 20 minutes. (ABTS/H_2O_2 solution: 1.0 µL 30% H_2O_2 in 10 mL ABTS stock).

10. Stop reaction by adding 50 µL 5N H_2SO_4 (optional), and determine O.D. at 410 nm.

11. The maximal phosphotyrosine signal is determined by subtracting the value of the negative controls from the positive controls. The percent inhibition of phosphotyrosine content for extract-containing wells is then calculated, after subtraction of the negative controls.

EXAMPLE 20: MET AUTOPHOSPHORYLATION ASSAY - ELISA

This assay determines Met tyrosine kinase activity by analyzing Met protein kinase levels on the Met receptor.

Reagents

a. HNTG (5X stock solution): Dissolve 23.83 g HEPES and 43.83 g NaCl in about 350 mL dH_2O. Adjust pH to 7.2 with HCl or NaOH, add 500 mL glycerol and 10 mL Triton X-100, mix, add dH_2O to 1 L total volume. To make 1 L of 1X working solution add 200 mL 5X stock solution to 800 mL dH_2O, check and adjust pH as necessary, store at 4 °C.

b. PBS (Dulbecco's Phosphate-Buffered Saline), Gibco Cat. # 450-1300EB (1X solution).

c. Blocking Buffer: in 500 mL dH_2O place 100 g BSA, 12.1 g Tris-pH7.5, 58.44 g NaCl and 10 mL Tween-20, dilute to 1 L total volume.

d. Kinase Buffer: To 500 mL dH_2O add 12.1 g TRIS pH7.2, 58.4 g NaCl, 40.7 g $MgCl_2$ and 1.9 g EGTA; bring to 1 L total volume with dH_2O.

e. PMSF (Phenylmethylsulfonyl fluoride), Sigma Cat. # P-7626, to 435.5 mg, add 100% ethanol to 25 mL total volume, vortex.

f. ATP (Bacterial Source), Sigma Cat. # A-7699, store powder at -20 °C; to make up solution for use, dissolve 3.31 mg in 1 mL dH_2O.

g. RC-20H HRPO Conjugated Anti-Phosphotyrosine, Transduction Laboratories Cat. # E120H.

h. Pierce 1-Step (TM) Turbo TMB-ELISA (3,3',5,5'-tetramethylbenzidine, Pierce Cat. # 34022.

i. H_2SO_4, add 1 mL conc. (18N) to 35 mL dH_2O.

j. TRIS HCL, Fischer Cat. # BP152-5; to 121.14 g of material, add 600 mL MilliQ H_2O, adjust pH to 7.5 (or 7.2) with HCl , bring volume to 1 L with MilliQ H_2O.

k. NaCl, Fischer Cat. # S271-10, make up 5M solution.

l. Tween-20, Fischer Cat. # S337-500.

m. Na_3VO_4, Fischer Cat. # S454-50, to 1.8 g material add 80 mL MilliQ H_2O, adjust pH to 10.0 with HCl or NaOH, boil in microwave, cool, check pH, repeat procedure until pH stable at 10.0, add MilliQ H_2O to 100 mL total volume, make 1 mL aliquots and store at -80 °C.

n. $MgCl_2$, Fischer Cat. # M33-500, make up 1M solution.

o. HEPES, Fischer Cat. # BP310-500, to 200 mL MilliQ H_2O, add 59.6 g material, adjust pH to 7.5, bring volume to 250 mL total, sterile filter.

p. Albumin, Bovine (BSA), Sigma Cat. # A-4503, to 30 grams material add sterile distilled water to make total volume of 300 mL, store at 4 °C.

q. TBST Buffer: to approx. 900 mL dH_2O in a 1 L graduated cylinder add 6.057 g TRIS and 8.766 g NaCl, when dissolved, adjust pH to 7.2 with HCl, add 1.0 mL Triton X-100 and bring to 1 L total volume with dH_2O.

r. Goat Affinity purified antibody Rabbit IgG (whole molecule), Cappel Cat. # 55641.

s. Anti h-Met (C-28) rabbit polyclonal IgG antibody, Santa Cruz Chemical Cat. # SC-161.

t. Transiently Transfected EGFR/Met chimeric cells (EMR) (Komada, et al., Oncogene, 8:2381-2390 (1993).

u. Sodium Carbonate Buffer, (Na_2CO_4, Fischer Cat. # S495): to 10.6 g material add 800 mL MilliQ H_2O, when dissolved adjust pH to 9.6 with NaOH, bring up to 1 L total volume with MilliQ H_2O, filter, store at 4 °C.

Procedure

All of the following steps are conducted at room temperature unless it is specifically indicated otherwise. All ELISA plate washing is by rinsing 4X with TBST.

A. EMR Lysis

This procedure can be performed the night before or immediately prior to the start of receptor capture.

1. Quick thaw lysates in a 37 °C waterbath with a swirling motion until the last crystals disappear.

2. Lyse cell pellet with 1X HNTG containing 1 mM PMSF. Use 3 mL of HNTG per 15 cm dish of cells. Add 1/ the calculated HNTG volume, vortex the tube for 1 min., add the remaining amount of HNTG, vortex for another min.

3. Balance tubes, centrifuge at 10,000x g for 10 min at 4 °C.

4. Pool supernatants, remove an aliquot for protein determination.

5. Quick freeze pooled sample in dry ice/ethanol bath. This step is performed regardless of whether lysate will be stored overnight or used immediately following protein determination.

6. Perform protein determination using standard bicinchoninic acid (BCA) method (BCA Assay Reagent Kit from Pierce Chemical Cat. # 23225).

ELISA Procedure

1. Coat Corning 96 well ELISA plates with 5 µg per well Goat anti-Rabbit antibody in Carbonate Buffer for a total well volume of 50 µl. Store overnight at 4 °C.

2. Remove unbound Goat anti-rabbit antibody by inverting plate to remove liquid.

3. Add 150 µL of Blocking Buffer to each well. Incubate for 30 min. at room temperature with shaking.

4. Wash 4X with TBST. Pat plate on a paper towel to remove excess liquid and bubbles.

5. Add 1µg per well of Rabbit anti-Met antibody diluted in TBST for a total well volume of 100 µl.

6. Dilute lysate in HNTG (90 µg lysate/100µl)

7. Add 100 µL of diluted lysate to each well. Shake at room temperature for 60 min.

8. Wash 4X with TBST. Pat on paper towel to remove excess liquid and bubbles.

9. Add 50 µL of 1X lysate buffer per well.

10. Dilute compounds/extracts 1:10 in 1X Kinase Buffer in a polypropylene 96 well plate.

11. Transfer 5.5 µL of diluted drug to ELISA plate wells. Incubate at room temperature with shaking for 20 min.

12. Add 5.5 µL of 60 µM ATP solution per well. Negative controls do not receive any ATP. Incubate at room temperature for 90 min., with shaking.

13. Wash 4X with TBST. Pat plate on paper towel to remove excess liquid and bubbles.

14. Add 100 µL per well of RC20 (1:3000 dilution in Blocking Buffer). Incubate 30 min. at room temperature with shaking.

15. Wash 4X with TBST. Pat plate on paper towel to remove excess liquid and bubbles.

16. Add 100 µL per well of Turbo-TMB. Incubate with shaking for 30-60 min.

17. Add 100 µL per well of 1M H_2SO_4 to stop reaction.

18. Read assay on Dynatech MR7000 ELISA reader. Test Filter = 450 nm, reference filter = 410 nm.

EXAMPLE 21: BIOCHEMICAL SRC ASSAY - ELISA

This assay is used to determine src protein kinase activity measuring phosphorylation of a biotinylated peptide as the readout.

Materials and Reagents:

a. Yeast transformed with src (Sugen, Inc., Redwood City, California).

b. Cell lysates: Yeast cells expressing src are pelleted, washed once with water, re-pelleted and stored at -80 °C until use.

c. N-terminus biotinylated EEEYEEYEEEYEEEYEEEY is prepared by standard procedures well known to those skilled in the art.

d. DMSO: Sigma, St. Louis, MO.

e. 96 Well ELISA Plate: Corning 96 Well Easy Wash, Modified flat Bottom Plate, Corning Cat. #25805-96.

f. NUNC 96-well V-bottom polypropylene plates for dilution of compounds: Applied Scientific Cat. # A-72092.

g. Vecastain ELITE ABC reagent: Vector, Burlingame, CA.

h. Anti-src (327) mab: Schizosaccharomyces Pombe was used to express recombinant Src (Superti-Furga, et al., EMBO J., 12:2625-2634; Superti-Furga, et al., Nature Biochem., 14:600-605). S. Pombe strain SP200 (h-s leul.32 ura4 ade210) was grown as described and transformations were pRSP expression plasmids were done by the lithium acetate method (Superti-Furga, supra). Cells were grown in the presence of 1 μM thiamine to repress expression from the nmtl promoter or in the absence of thiamine to induce expression.

i. Monoclonal anti-phosphotyrosine, UBI 05-321 (UB40 may be used instead).

j. Turbo TMB-ELISA peroxidase substrate: Pierce Chemical.

Buffer Solutions:

a. PBS (Dulbecco's Phosphate-Buffered Saline): GIBCO PBS, GIBCO Cat. # 450-1300EB.

b. Blocking Buffer: 5% Non-fat milk (Carnation) in PBS.

c Carbonate Buffer: Na_2CO_4 from Fischer, Cat. # S495, make up 100 mM stock solution.

d. Kinase Buffer: 1.0 mL (from 1M stock solution) $MgCl_2$; 0.2 mL (from a 1M stock solution) $MnCl_2$; 0.2 mL (from a 1M stock solution) DTT; 5.0 mL (from a 1M stock solution) HEPES; 0.1 mL TX-100; bring to 10 mL total volume with MilliQ H_2O.

e. Lysis Buffer: 5.0 HEPES (from 1M stock solution.); 2.74 mL NaCl (from 5M stock solution); 10 mL glycerol; 1.0 mL TX-100; 0.4 mL EDTA (from a 100 mM stock solution); 1.0 mL PMSF (from a 100 mM stock solution); 0.1 mL Na_3VO_4 (from a 0.1 M stock solution); bring to 100 mL total volume with MilliQ H_2O.

f. ATP: Sigma Cat. # A-7699, make up 10 mM stock solution (5.51 mg/mL).

g TRIS-HCl: Fischer Cat. # BP 152-5, to 600 mL MilliQ H_2O add 121.14 g material, adjust pH to 7.5 with HCl, bring to 1 L total volume with MilliQ H_2O.

h. NaCl: Fischer Cat. # S271-10, Make up 5M stock solution with MilliQ H_2O.

Na_3VO_4: Fischer Cat. # S454-50; to 80 mL MilliQ H_2O, add 1.8 g material; adjust pH to 10.0 with HCl or NaOH; boil in a microwave; cool; check pH, repeat pH adjustment until

pH remains stable after heating/cooling cycle; bring to 100 mL total volume with MilliQ H_2O; make 1 mL aliquots and store at -80 °C.

j. $MgCl_2$: Fischer Cat. # M33-500, make up 1M stock solution with MilliQ H_2O.

k. HEPES: Fischer Cat. # BP 310-500; too 200 mL MilliQ H_2O, add 59.6 g material, adjust pH to 7.5, bring to 250 mL total volume with MilliQ H_2O, sterile filter (1M stock solution).

l. TBST Buffer: TBST Buffer: To 900 mL dH_2O add 6.057 g TRIS and 8.766 g NaCl; adjust pH to 7.2 with HCl, add 1.0 mL Triton-X100; bring to 1 L total volume with dH_2O.

m. $MnCl_2$: Fischer Cat. # M87-100, make up 1M stock solution with MilliQ H2O.

n. DTT; Fischer Cat. # BP172-5.

o. TBS (TRIS Buffered Saline): to 900 mL MilliQ H_2O add 6.057 g TRIS and 8.777 g NaCl; bring to 1 L total volume with MilliQ H2O.

p. Kinase Reaction Mixture: Amount per assay plate (100 wells): 1.0 mL Kinase Buffer, 200 µg GST- , bring to final volume of 8.0 mL with MilliQ H_2O.

q. Biotin labeled EEEYEEYEEEYEEEYEEEY: Make peptide stock solution (1mM, 2.98 mg/mL) in water fresh just before use.

r. Vectastain ELITE ABC reagent: To prepare 14 mL of working reagent, add 1 drop of reagent A to 15 mL TBST and invert tube several times to mix. Then add 1 drop of reagent B. Put tube on orbital shaker at room temperature and mix for 30 minutes.

Procedures:

A. Preparation Of Src Coated Elisa Plate

1. Coat ELISA plate with 0.5 µg/well anti-src mab in 100 µL of pH 9.6 sodium carbonate buffer at 4 °C overnight.

2. Wash wells once with PBS.

3. Block plate with 0.15 mL 5% milk in PBS for 30 min. at room temperature.

4. Wash plate 5X with PBS.

5. Add 10 µg/well of src transformed yeast lysates diluted in Lysis Buffer (0.1 mL total volume per well). (Amount of lysate may vary between batches.) Shake plate for 20 minutes at room temperature.

B. Preparation Of Phosphotyrosine Antibody-Coated Elisa Plate

1. 4G10 plate: coat 0.5 μg/well 4G10 in 100 μL PBS overnight at 4 °C and block with 150 μL of 5% milk in PBS for 30 minutes at room temperature.

C. Kinase assay procedure

1. Remove unbound proteins from step 1-7, above, and wash plates 5X with PBS.

2. Add 0.08 mL Kinase Reaction Mixture per well (containing 10 μL of 10X Kinase Buffer and 10 μM (final concentration) biotin-EEEYEEYEEEYEEEYEEEY per well diluted in water.

3. Add 10 μL of compound diluted in water containing 10% DMSO and pre-incubate for 15 minutes at room temperature.

4. Start kinase reaction by adding 10 μL/well of 0.05 mM ATP in water (5 μM ATP final).

5. Shake ELISA plate for 15 min. at room temperature.

6. Stop kinase reaction by adding 10 μL of 0.5 M EDTA per well.

7. Transfer 90 μL supernatant to a blocked 4G10 coated ELISA plate from section B, above.

8. Incubate for 30 min. while shaking at room temperature.

9. Wash plate 5X with TBST.

10. Incubate with Vectastain ELITE ABC reagent (100 μl/well) for 30 min. at room temperature.

11. Wash the wells 5X with TBST.

12. Develop with Turbo TMB.

EXAMPLE 22: BIOCHEMICAL LCK ASSAY - ELISA

This assay is used to determine lck protein kinase activities measuring phosphorylation of GST- as the readout.

Materials and Reagents:

a. Yeast transformed with lck. Schizosaccharomyces Pombe was used to express recombinant Lck (Superti-Furga, et al., EMBO J, 12:2625-2634; Superti-Furga, et al.,

Nature Biotech., 14:600-605). S. Pombe strain SP200 (h-s leul.32 ura4 ade210) was grown as described and transformations with pRSP expression plasmids were done by the lithium acetate method (Superti-Furga, supra). Cells were grown in the presence of 1 μM thiamine to induce expression.

b. Cell lysates: Yeast cells expressing lck are pelleted, washed once in water, re-pelleted and stored frozen at -80 °C until use.

c. GST- : DNA encoding for GST- fusion protein for expression in bacteria obtained from Arthur Weiss of the Howard Hughes Medical Institute at the University of California, San Francisco. Transformed bacteria were grown overnight while shaking at 25 °C. GST- was purified by glutathione affinity chromatography, Pharmacia, Alameda, CA.

d. DMSO: Sigma, St. Louis, MO.

e. 96-Well ELISA plate: Corning 96 Well Easy Wash, Modified Flat Bottom Plate, Corning Cat. #25805-96.

f. NUNC 96-well V-bottom polypropylene plates for dilution of compounds: Applied Scientific Cat. # AS-72092.

g. Purified Rabbit anti-GST antiserum: Amrad Corporation (Australia) Cat. #90001605.

h. Goat anti-Rabbit-IgG-HRP: Amersham Cat. # V010301

i. Sheep ant-mouse IgG (H+L): Jackson Labs Cat. # 5215-005-003.

j. Anti-Lck (3A5) mab: Santa Cruz Biotechnology Cat # sc-433.

k. Monoclonal anti-phosphotyrosine UBI 05-321 (UB40 may be used instead).

Buffer solutions:

a. PBS (Dulbecco's Phosphate-Buffered Saline) 1X solution: GIBCO PBS, GIBCO Cat. # 450-1300EB.

b. Blocking Buffer: 100 g. BSA, 12.1 g. TRIS-pH7.5, 58.44 g NaCl, 10 mL Tween-20, bring up to 1 L total volume with MilliQ H_2O.

c. Carbonate Buffer: Na_2CO_4 from Fischer, Cat. # S495; make up 100 mM solution with MilliQ H_2O.

d. Kinase Buffer: 1.0 mL (from 1M stock solution) $MgCl_2$; 0.2 mL (from a 1M stock solution) $MnCl_2$; 0.2 mL (from a 1M stock solution) DTT; 5.0 mL (from a 1M stock solution) HEPES; 0.1 mL TX-100; bring to 10 mL total volume with MilliQ H_2O.

e. Lysis Buffer: 5.0 HEPES (from 1M stock solution.); 2.74 mL NaCl (from 5M stock solution); 10 mL glycerol; 1.0 mL TX-100; 0.4 mL EDTA (from a 100 mM stock solution); 1.0 mL PMSF (from a 100 mM stock solution); 0.1 mL Na_3VO_4 (from a 0.1 M stock solution); bring to 100 mL total volume with MilliQ H_2O.

f. ATP: Sigma Cat. # A-7699, make up 10 mM stock solution (5.51 mg/mL).

g TRIS-HCl: Fischer Cat. # BP 152-5, to 600 mL MilliQ H_2O add 121.14 g material, adjust pH to 7.5 with HCl, bring to 1 L total volume with MilliQ H_2O.

h. NaCl: Fischer Cat. # S271-10, Make up 5M stock solution with MilliQ H_2O.

i Na_3VO_4: Fischer Cat. # S454-50; to 80 mL MilliQ H_2O, add 1.8 g material; adjust pH to 10.0 with HCl or NaOH; boil in a microwave; cool; check pH, repeat pH adjustment until pH remains stable after heating/cooling cycle; bring to 100 mL total volume with MilliQ H_2O; make 1 mL aliquots and store at -80 °C.

j. $MgCl_2$: Fischer Cat. # M33-500, make up 1M stock solution with MilliQ H_2O.

k. HEPES: Fischer Cat. # BP 310-500; to 200 mL MilliQ H_2O, add 59.6 g material, adjust pH to 7.5, bring to 250 mL total volume with MilliQ H_2O, sterile filter (1M stock solution).

l. Albumin, Bovine (BSA), Sigma Cat. # A4503; to 150 mL MilliQ H_2O add 30 g material, bring 300 mL total volume with MilliQ H_2O, filter through 0.22 µm filter, store at 4 °C.

m. TBST Buffer: To 900 mL dH_2O add 6.057 g TRIS and 8.766 g NaCl; adjust pH to 7.2 with HCl, add 1.0 mL Triton-X100; bring to 1 L total volume with dH_2O.

n. $MnCl_2$: Fischer Cat. # M87-100, make up 1M stock solution with MilliQ H_2O.

o. DTT; Fischer Cat. # BP172-5.

p. TBS (TRIS Buffered Saline): to 900 mL MilliQ H_2O add 6.057 g TRIS and 8.777 g NaCl; bring to 1 L total volume with MilliQ H_2O.

q Kinase Reaction Mixture: Amount per assay plate (100 wells): 1.0 mL Kinase Buffer, 200 µg GST- , bring to final volume of 8.0 mL with MilliQ H_2O.

Procedures:

A. Preparation Of Lck Coated Elisa Plate

1. Coat 2.0 µg/well Sheep anti-mouse IgG in 100 µL of pH 9.6 sodium carbonate buffer at 4 °C overnight.

2. Wash well once with PBS.

3. Block plate with 0.15 mL of blocking Buffer for 30 min. at room temp.

4. Wash plate 5X with PBS.

5. Add 0.5 µg/well of anti-lck (mab 3A5) in 0.1 mL PBS at room temperature for 1-2 hours.

6. Wash plate 5X with PBS.

7. Add 20 µg/well of lck transformed yeast lysates diluted in Lysis Buffer (0.1 mL total volume per well). (Amount of lysate may vary between batches) Shake plate at 4 °C overnight to prevent loss of activity.

B. Preparation Of Phosphotyrosine Antibody-Coated Elisa Plate

1. UB40 plate: 1.0 µg/well UB40 in 100 µL of PBS overnight at 4 °C and block with 150 µL of Blocking Buffer for at least 1 hour.

C. Kinase Assay Procedure

1. Remove unbound proteins from step 1-7, above, and wash plates 5X with PBS.

2. Add 0.08 mL Kinase Reaction Mixture per well (containing 10 µL of 10X Kinase Buffer and 2 µg GST- per well diluted with water).

3. Add 10 µL of compound diluted in water containing 10% DMSO and pre-incubate for 15 minutes at room temperature.

4. Start kinase reaction by adding 10 µL/well of 0.1 mM ATP in water (10 µM ATP final).

5. Shake ELISA plate for 60 min. at room temperature.

6. Stop kinase reaction by adding 10 µL of 0.5 M EDTA per well.

7. Transfer 90 µL supernatant to a blocked 4G10 coated ELISA plate from section B, above.

8. Incubate while shaking for 30 min. at room temperature.

9. Wash plate 5X with TBST.

10. Incubate with Rabbit anti-GST antibody at 1:5000 dilution in 100 µL TBST for 30 min. at room temperature.

11. Wash the wells 5X with TBST.

12. Incubate with Goat anti-Rabbit-IgG-HRP at 1:20,000 dilution in 100 µL of TBST for 30 min. at room temperature.

13. Wash the wells 5X with TBST.

14. Develop with Turbo TMB.

EXAMPLE 23: ASSAY MEASURING PHOSPHORYLATING FUNCTION OF RAF

The following assay reports the amount of RAF-catalyzed phosphorylation of its target protein MEK as well as MEK's target MAPK. The RAF gene sequence is described in Bonner et al., 1985, Molec. Cell. Biol. 5: 1400-1407, and is readily accessible in multiple gene sequence data banks. Construction of the nucleic acid vector and cell lines utilized for this portion of the invention are fully described in Morrison et al., 1988, Proc. Natl. Acad. Sci. USA 85: 8855-8859.

Materials and Reagents

1. *Sf9 (Spodoptera frugiperda)* cells; GIBCO-BRL, Gaithersburg, MD.

2. RIPA buffer: 20 mM Tris/HC1 pH 7.4, 137 mM NaCl, 10% glycerol, 1 mM PMSF, 5 mg/L Aprotenin, 0.5 % Triton X-100;

3. Thioredoxin-MEK fusion protein (T-MEK): T-MEK expression and purification by affinity chromatography were performed according to the manufacturer's procedures. Catalog# K 350-01 and R 350-40, Invitrogen Corp., San Diego, CA

4. His-MAPK (ERK 2); His-tagged MAPK was expressed in XL1 Blue cells transformed with pUC18 vector encoding His-MAPK. His-MAPK was purified by Ni-affinity chromatography. Cat# 27-4949-01, Pharmacia, Alameda, CA, as described herein.

5. Sheep anti mouse IgG: Jackson laboratories, West Grove, PA. Catalog, # 515-006-008, Lot# 28563

6. RAF-1 protein kinase specific antibody: URP2653 from UBI.

7. Coating buffer: PBS; phosphate buffered saline, GIBCO-BRL, Gaithersburg, MD

8. Wash buffer: TBST - 50 mM Tris/HCL pH 7.2, 150 mM NaCl, 0.1 % Triton X-100

9. Block buffer: TBST, 0.1 % ethanolamine pH 7.4

10. DMSO, Sigma, St. Louis, MO

11. Kinase buffer (KB): 20 mM HEPES/HC1 pH 7.2, 150 mM NaCl, 0.1 % Triton X-100, 1 mM PMSF, 5 mg/L Aprotenin, 75 mM sodium ortho vanadate, 0.5 MM DTT and 10 mM $MgCl_2$.

12. ATP mix: l00 mM $MgCl_2$, 300 mM ATP, 10 mCi ^{33}P ATP (Dupont-NEN)/mL.

13 Stop solution: 1 % phosphoric acid; Fisher, Pittsburgh, PA.

14. Wallac Cellulose Phosphate Filter mats; Wallac, Turku, Finnland.

15. Filter wash solution: 1 % phosphoric acid, Fisher, Pittsburgh, PA.

16. Tomtec plate harvester, Wallac, Turku, Finnland.

17. Wallac beta plate reader # 1205, Wallac, Turku, Finnland.

18. NUNC 96-well V bottom polypropylene plates for compounds Applied Scientific Catalog # AS-72092.

Procedure

All of the following steps were conducted at room temperature unless specifically indicated.

1. ELISA plate coating: ELISA wells are coated with 100 mL of Sheep anti mouse affinity purified antiserum (l mg/l00 mL coating buffer) over night at 4 °C. ELISA plates can be used for two weeks when stored at 4 °C.

2. Invert the plate and remove liquid. Add 100 mL of blocking solution and incubate for 30 min.

3. Remove blocking solution and wash four times with wash buffer. Pat the plate on a paper towel to remove excess liquid.

4. Add 1 mg of antibody specific for RAF-1 to each well and incubate for 1 hour. Wash as described in step 3.

5. Thaw lysates from RAS/RAF infected Sf9 cells and dilute with TBST to 10 mg/100 mL. Add 10 mg of diluted lysate to the wells and incubate for 1 hour. Shake the plate during incubation. Negative controls receive no lysate. Lysates from RAS/RAF

infected Sf9 insect cells are prepared after cells are infected with recombinant baculoviruses at a MOI of 5 for each virus, and harvested 48 hours later. The cells are washed once with PBS and lysed in RIPA buffer. Insoluble material is removed by centrifugation (5 min at 10 000 x g). Aliquots of lysates are frozen in dry ice/ethanol and stored at -80 °C until use.

6. Remove non-bound material and wash as outlined above (step 3).

7. Add 2 mg of T-MEK and 2 mg of His-MAEPK per well and adjust the volume to 40 mL with kinase buffer. Methods for purifying T-MEK and MAPK from cell extracts are provided herein by example.

8. Pre-dilute compounds (stock solution 10 mg/mL DMSO) or extracts 20 fold in TBST plus 1% DMSO. Add 5 mL of the pre-diluted compounds/extracts to the wells described in step 6. Incubate for 20 min. Controls receive no drug.

9. Start the kinase reaction by addition of 5 mL ATP mix; Shake the plates on an ELISA plate shaker during incubation.

10. Stop the kinase reaction after 60 min by addition of 30 mL stop solution to each well.

11. Place the phosphocellulose mat and the ELISA plate in the Tomtec plate harvester. Harvest and wash the filter with the filter wash solution according to the manufacturers recommendation. Dry the filter mats. Seal the filter mats and place them in the holder. Insert the holder into radioactive detection apparatus and quantify the radioactive phosphorous on the filter mats.

Alternatively, 40 mL aliquots from individual wells of the assay plate can be transferred to the corresponding positions on the phosphocellulose filter mat. After air drying the filters, put the filters in a tray. Gently rock the tray, changing the wash solution at 15 min intervals for 1 hour. Air-dry the filter mats. Seal the filter mats and place them in a holder suitable for measuring the radioactive phosphorous in the samples. Insert the holder into a detection device and quantify the radioactive phosphorous on the filter mats.

EXAMPLE 24: CDK2/CYCLIN A - INHIBITION ASSAY

This assay analyzes the protein kinase activity of CDK2 in exogenous substrate.

Reagents:

A. Buffer A (80 mM Tris (pH 7.2), 40 mM $MgCl_2$): 4.84 G. Tris (F.W. =121.1 g/mol), 4.07 g. $MgCl_2$ (F.W.=203.31 g/mol) dissolved in 500 mL H_2O. Adjust pH to 7.2 with HCl.

B. Histone H1 solution (0.45 mg/mL Histone H1 and 20 mM HEPES pH 7.2 (pH 7.4 is OK): 5 mg Histone H1 (Boehinger Mannheim) in 11.111 mL 20 mM HEPES pH 7.2 (477 mg HEPES (F.W.= 238.3 g/mol) dissolved in 100 mL ddH2O, stored in 1 mL aliquots at -80 °C.

C. ATP solution (60 µM ATP, 300 µg/mL BSA, 3 mM DTT): 120 µL 10 mM ATP, 600 µL 10 mg/mL BSA to 20 mL, stored in 1 mL aliquots at -80 °C.

D. CDK2 solution: cdk2/cyclin A in 10 mM HEPES pH 7.2, 25 mM NaCl, 0.5 mM DTT, 10% glycerol, stored in 9 µL aliquots at -80 °C.

Description of Assay:

1. Prepare solutions of inhibitors at three times the desired final assay concentration in ddH_2O/15 % DMSO by volume.

2. Dispense 20 µL of inhibitors to wells of polypropylene 96-well plates (or 20 µL 15% DMSO for positive and negative controls).

3. Thaw Histone H1 solution (1 mL/plate), ATP solution (1 mL/plate plus 1 aliquot for negative control), and CDK2 solution (9 µL/plate). Keep CDK2 on ice until use. Aliquot CDK2 solution appropriately to avoid repeated freeze-thaw cycles.

4. Dilute 9 µL CDK2 solution into 2.1 mL Buffer A (per plate). Mix. Dispense 20 µL into each well.

5. Mix 1 mL Histone H1 solution with 1 mL ATP solution (per plate) into a 10 mL screw cap tube. Add ^{33}P ATP to a concentration of 0.15 µCi/20 µL (0.15 µCi/well in assay). Mix carefully to avoid BSA frothing. Add 20 µL to appropriate wells. Mix plates on plate shaker. For negative control, mix ATP solution with an equal amount of 20 mM HEPES pH 7.2 and add ^{33}P ATP to a concentration of 0.15 µCi/20 µL solution. Add 20 µL to appropriate wells.

6. Let reactions proceed for 60 minutes.

7. Add 35 µL 10% TCA to each well. Mix plates on plate shaker.

8. Spot 40 µL of each sample onto P30 filter mat squares. Allow mats to dry (approx. 10-20 minutes).

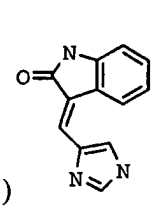
9 Wash filter mats 4 X 10 minutes with 250 mL 1% phosphoric acid (10 mL phosphoric acid per liter ddH_2O).

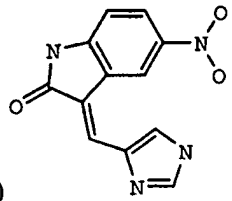
10. Count filter mats with beta plate reader.

Table 15 shows the results of biological assays of representative imidazoyl 2-indolinones of this invention. The compounds are listed following the Table. IC_{50} generally refers to that amount of a compound needed to effect a 50% change in the activity of a PK with respect to a control in which no compound is present. With regard to the tests in the table, the 50% change being evaluated is a 50% inhibition of PK activity by a compound of this invention over the activity of a control in the absence of any compound.

TABLE 15

Compounds	Corresponding Kinase Inhibitory Activity IC_{50} (:9)
II-001	<0.78
II-002	<0.78
II-003	<0.78
II-004	<0.78
II-005	<0.78
II-006	1.1
II-007	2.4
II-008	7.7
II-009	9.3


(II-001)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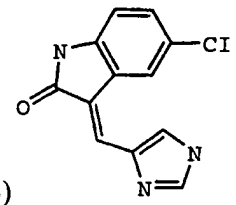
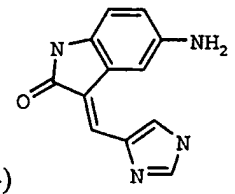
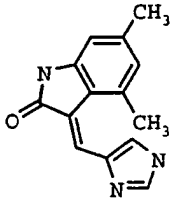
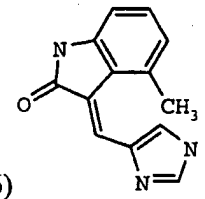
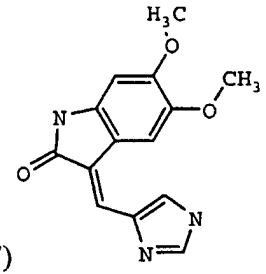
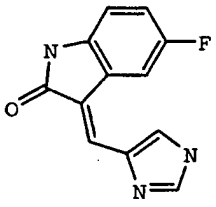

(II-003)

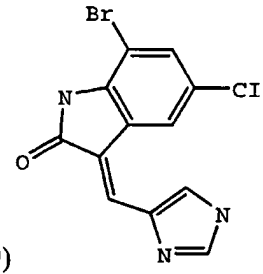

(II-004)


(II-005)


(II-006)


(II-007)


(II-008)


(II-009)

EXAMPLE 25: XENOGRAFT ANIMAL MODELS

The ability of human tumors to grow as xenografts in athymic mice (*e.g.*, Balb/c, nu/nu) provides a useful in vivo model for studying the biological response to therapies for human tumors. Since the first successful xenotransplantation of human tumors into athymic mice, (Rygaard and Povlsen, 1969, Acta Pathol. Microbial. Scand. 77:758-760), many different human tumor cell lines (*e.g.*, mammary, lung, genitourinary, gastro-intestinal, head and neck, glioblastoma, bone, and malignant melanomas) have been transplanted and successfully grown in nude mice. The following assays may be used to determine the level of activity, specificity and effect of the different compounds of the present invention. Three general types of assays are useful for evaluating compounds: cellular/catalytic, cellular/biological and in vivo. The object of the cellular/catalytic assays is to determine the effect of a compound on the ability of a TK to phosphorylate tyrosines on a known substrate in a cell. The object of the cellular/biological assays is to determine the effect of a compound on the biological response stimulated by a TK in a

cell. The object of the in vivo assays is to determine the effect of a compound in an animal model of a particular disorder such as cancer.

Suitable cell lines for subcutaneous xenograft experiments include C6 cells (glioma, ATCC # CCL 107), A375 cells (melanoma, ATCC # CRL 1619), A431 cells (epidermoid carcinoma, ATCC # CRL 1555), Calu 6 cells (lung, ATCC # HTB 56), PC3 cells (prostate, ATCC # CRL 1435) and NIH 3T3 fibroblasts genetically engineered to overexpress EGFR, PDGFR, IGF-1R or any other test kinase. The following protocol can be used to perform xenograft experiments:

Female athymic mice (BALB/c, nu/nu) are obtained from Simonsen Laboratories (Gilroy, CA). All animals are maintained under clean-room conditions in Micro-isolator cages with Alpha-dri bedding. They receive sterile rodent chow and water ad libitum.

Cell lines are grown in appropriate medium (for example, MEM, DMEM, Ham's F10, or Ham's F12 plus 5% - 10% fetal bovine serum (FBS) and 2 mM glutamine (GLN)). All cell culture media, glutamine, and fetal bovine serum are purchased from Gibco Life Technologies (Grand Island, NY) unless otherwise specified. All cells are grown in a humid atmosphere of 90-95% air and 5-10% CO_2 at 37 °C. All cell lines are routinely subcultured twice a week and are negative for mycoplasma as determined by the Mycotect method (Gibco).

Cells are harvested at or near confluency with 0.05% Trypsin-EDTA and pelleted at 450 x g for 10 min. Pellets are resuspended in sterile PBS or media (without FBS) to a particular concentration and the cells are implanted into the hindflank of the mice (8 - 10 mice per group, 2 - 10 x 10^6 cells/animal). Tumor growth is measured over 3 to 6 weeks using venier calipers. Tumor volumes are calculated as a product of length x width x height unless otherwise indicated. P values are calculated using the Students t-test. Test compounds in 50 - 100 µL excipient (DMSO, or VPD:D5W) was delivered by IP injection at different concentrations generally starting at day one after implantation.

EXAMPLE 26: TUMOR INVASION MODEL

The following tumor invasion model has been developed and maybe used for the evaluation of therapeutic value and efficacy of the compounds identified to selectively inhibit KDR/FLK-1 receptor.

Procedure

8 week old nude mice (female) (Simonsen Inc.) were used as experimental animals. Implantation of tumor cells was performed in a laminar flow hood. For anesthesia, Xylazine/Ketamine Cocktail (100 mg/kg ketamine and 5 mg/kg Xylazine) are administered intraperitoneally. A midline incision is done to expose the abdominal cavity (approximately 1.5 cm in length) to inject 10^7 tumor cells in a volume of 100 µL medium. The cells are injected either into the duodenal lobe of the pancreas or under the serosa of the colon. The peritoneum and muscles are closed with a 6-0 silk continuous suture and the skin was closed by using wound clips. Animals were observed daily.

Analysis

After 2-6 weeks, depending on gross observations of the animals, the mice are sacrificed, and the local tumor metastases, to various organs (lung, liver, brain, stomach, spleen, heart, muscle) are excised and analyzed (measurements of tumor size, grade of invasion, immunochemistry, and in situ hybridization).

EXAMPLE 27: MEASUREMENT OF CELL TOXICITY

Therapeutic compounds should be more potent in inhibiting protein kinase activity than in exerting a cytotoxic effect. A measure of the effectiveness and cell toxicity of a compound can be obtained by determining the therapeutic index: IC_{50}/LD_{50}. IC_{50}, the dose required to achieve 50% inhibition, can be measured using standard techniques such as those described herein. LD_{50}, the dosage which results in 50% toxicity, can also be measured by standard techniques (Mossman, 1983, J. Immunol. Methods, 65:55-63), by measuring the amount of LDH released (Korzeniewski and Callewaert, 1983, J. Immunol. Methods, 64:313; Decker and Lohmann-Matthes, 1988, J. Immunol. Methods, 115:61), or by measuring the lethal dose in animal models. Compounds with a large therapeutic index are preferred. The therapeutic index should be greater than 2, preferably at least 10, more preferably at least 50.

EXAMPLE 28: Demonstration of Inhibition of Vascular Endothelial Growth Factor-Stimulated Cell Proliferation

The effect of several indolinone compounds (Compound AV-002, Compound AV-003, and Compound AV-004) on VEGF-stimulated cell proliferation of human umbilical vein endothelial cells (HUVEC) is described. The indolinone compounds prevent the phosphorylation of substrate molecules by the activated receptor tyrosine kinase and thereby block signal transduction and the resulting cell proliferation.

The three compounds evaluated inhibited VEGF-stimulation of HUVECs with differing effectiveness and specificity. In this example, Compound AV-003 was more potent than Compound AV-002, while Compound AV-004 apparently inhibited VEGF-stimulated proliferation of HUVEC through nonspecific antiproliferative mechanisms, similar to those observed with doxorubicin, a reference compound.

The cellular mechanism of action of Compound AV-002 and Compound AV-003 can be explained by inhibition of the receptor tyrosine kinases and thereby signal transduction to the cell nucleus which is necessary for the start of cell proliferation. However, the inventors do not wish to be restricted to this explanation.

Materials and Methods:

The cells used were HUV-EC-C (umbilical cord, human) obtained from the ATCC. The cells were cultivated in MEM (Sigma, M5650) + 10% FCS (Gibco Brl) + bullet kit and passaged once/week. Only early passages (up to passage 10) were used. The cells were isolated from cell culture flasks by incubation with trypsin/EDTA.

The test compounds were dissolved at 40 mM in DMSO and stored at 4 °C. The reference compound doxorubicin was dissolved at 2 mM in A. dest.

The control groups were cells without VEGF and without substances. The test groups were the corresponding groups with additions of serial dilutions of test substances.

Experimental Conditions *in vitro*: (1) TCA (10%): 10 g TCA was dissolved in 90 g A. dest.; (2) Acetic acid (1%, Merck): 1 mL acetic acid was dissolved in 99 mL of A. dest.; (3) SRB solution (0.4%, Sigma): 2 g sulforhodamin B was dissolved in 500 mL acetic acid (1%); (4) TRIS buffer (10 mM): 1.211 g/L TRIS was dissolved in A. dest. and titrated to pH 10.5 with NaOH (1 M); (5) VEGF stock solution (10 µg/mL): 10 µg VEGF

were dissolved in 1000 µL A. dest. Further dilution was done directly in MEM + 1% FCS (2241 µL medium + 9 µL VEGF stock solution).

Test procedure: $5x10^3$ cells/well in 100 µL MEM + 1% FCS were seeded in a 96-well microliter plate (37 °C; 5% CO_2 95% relative humidity). After overnight incubation, the test compounds in 50 µL MEM were added to the wells. For each dilution, 8 wells were performed. 5 min later, 50 µL VEGF (0.04 µg/mL) was added to half of the wells; 50 µL medium was added to the other half of the wells. Incubation proceeded for 3 days. The medium was removed and 100 µL TCA (10%) was added and incubated for 1 h at 4 °C. Wells were rinsed three times with 200 µL A. dest. Sulforhodamin B (SRB) solution (0.4% in 1% acetic acid) was added for 1 h at RT (100 µL/well). Wells were rinsed three times with 200 µL acetic acid 1%. The protein-bound dye was solubilized in 200 µL/well TRIS ph 10.5 with gentle shaking.

Concentrations of the compound in the *in vitro* system: serial dilutions were done in medium (MEM + 1% FCS). For the highest concentration, 576 µL medium + 24 µL from 40 mM substance stock solution were combined. For the next dilution step, 120 µL from the preceding dilution was added to 480 µL medium. Further serial dilution was done similarly, each time using 120 µL from the preceding dilution step.

For the highest Doxorubicin concentration, 480 µL medium + 120 µL from the 2 mM stock solution were combined. For each subsequent dilution step, 120 µL from the preceding dilution was added to 480 µL medium.

In the micro titer plates, the serial dilution series is finally diluted fourfold: test compounds (0.00512 - 0.0256 - 0.128 - 0.64 - 3.2 - 16 - 80 - 400 µM); doxorubicin (0.000256 - 0.00128 - 0.0064 - 0.032 - 0.16 - 0.8 - 4 - 20 - 100 µM).

Observations and Measurements:

The extinction value was measured for each well using an Immuno Reader NJ 2000 AT 570 nm. For Compound AV-004 and doxorubicin, the extinction value in the 300 µM group unexpectedly increased compared to the 80 µM group. Usually higher substance concentrations give rise to higher cell toxicity and therefor to lower extinction values. This phenomenon is due to the color of residual Compound AV-003 or doxorubicin inside the well and not to protein-bound SRB.

Evaluation of Data:

For each dilution and control, the mean extinction value of the 4 wells and the standard deviation was calculated (Microsoft Excel 5.0).

The IC_{50} was calculated with the equation "IC50 - 4 parameter logistic" (start at 0, defined end) out of the software program GraFit 3.0 (Erithacus Software Ltd., Staines, UK).

Inhibition of VEGF-stimulation is calculated according to the following formula,

$$\%\ inhibition = \left(100 - \left[\frac{Ex\ SU + PDGF - Ex\ SU - PDGF}{Ex\ control + PDGF - Ex\ control - PDGF} x\ 100\right]\right)\%$$

when PDGF is replaced by VEGF:

Ex = mean extinction of the respective group

Results:

To determine the appropriate concentration range for the test on inhibition of VEGF-stimulation for each compound a simple IC_{50} estimation (in the absence of additional VEGF) was done. 400 µM was the highest concentration (for the least toxic substance) tolerated by the cells. Therefore, this was the highest concentration tested in combination with VEGF.

The three compounds inhibited VEGF-stimulation of HUVECs with different potency and specificity (Table 1). The order was Compound AV-003 > Compound AV-002 > Compound AV-004. At 0.64 µM, the inhibition was nearly complete with Compound AV-003. Compound AV-002 gave a similar picture at a 5-fold higher concentration. The effects of Compound AV-004, in this example, seem to be more nonspecific, because inhibition occurred at concentrations at which inhibition of the unstimulated control also occurred.

A comparison of the inhibitory IC_{50} of VEGF-stimulated cells with the inhibitory IC_{50} of unstimulated cells (Table 1) shows a clear difference in inhibition when Compound AV-002 or Compound AV-003 is tested (IC_{50} for inhibition of VEGF-stimulated < IC_{50} for inhibition of unstimulated). However, tests with Compound AV-004 show no difference, or even an inverse difference in inhibition (IC_{50} for inhibition of VEGF-

stimulated > IC_{50} for inhibition of unstimulated). Doxorubicin (cytotoxic control) also showed a similar inhibition pattern on stimulated and unstimulated cells.

EXAMPLE 29: Demonstration of Inhibition of Platelet-Derived Growth Factor-Stimulated Cell Proliferation

The effect of the indolinone compounds of the invention (Compound AV-002, Compound AV-003, and Compound AV-004) on PDGF-stimulated cell proliferation of rat smooth muscle cells was studied using A10 cells (American Type Culture Collection. A10 cells are embryonic thoracic aorta smooth muscle cells from a DB1X rat. The three compounds show different levels of inhibition of PDGF-stimulation of A10 cells. This is particularly evident when the compounds are preincubated with the cells prior to PDGF addition. Doxorubicin was used as a reference compound.

Compound AV-002, Compound AV-003, and Compound AV-004 are able to inhibit PDGF-stimulated proliferation of (rat) aortic smooth muscle cells. In this example, Compound AV-004 was more potent than Compound AV-002, especially in the preincubation experiments, while Compound AV-003 apparently inhibited cell proliferation through a nonspecific mechanism.

The cellular mechanism of action of Compound AV-002 and Compound AV-004 can be explained by the inhibition of receptor tyrosine kinases and thereby signal transduction to the cell nucleus, which is a requisite for cell proliferation. However, the inventors should not be held to one possible explanation of the data. In this example, the proliferation data suggest that Compound AV-004 should be the most potent inhibitor of PDGF receptor tyrosine kinase.

Materials and Methods:

A10 cells (embryonic thoracic aorta smooth muscle cells, DB1X rat) were obtained from the ATCC. The cells were cultivated in MEM (Sigma, M5650) + 10% FCS (Gibco BRL) and were passaged once/week. The cells were isolated from cell culture flasks by incubation with trypsin/EDTA.

Test Compounds (Compound AV-002, Compound AV-003, and Compound AV-004) and the reference compound, doxorubicin, were prepared and stored as described in Example 28. The same experimental controls are included as in Example 28.

Experimental conditions *in vitro* are as described in Example 28, except that PDGF was substituted for VEGF. PDGF stock solution (100 µg/mL; Pepro Tech): 10 µg PDGF were dissolved in 100 µL A. dest. Further dilution was done directly in MEM + 5% FCS (2241 µL medium + 9 µL PDGF stock solution).

Test procedures were as in Example I except that after the test compounds were added to the wells, 50 µL PDGF, not VEGF, (0.4 µg/mL) was added to half of the wells and 50 µL medium was added to the other half, 5 min or 24 h later. The rest of the experimental protocol was the same as in Example 28.

Concentrations of the compound in the *in vitro* system are as described previously in Example 28.

Observations and Measurements:

Similarly to Example 28, the extinction value was measured for each well using an Immuno Reader NJ 2000 at 570 nm. For Compound AV-003, the extinction value in the 400 µM group unexpectedly increased compared to the 80 µM group. Usually higher compound concentrations give rise to higher cell toxicity and therefor to lower extinction values. This phenomenon is due to the color of residual Compound AV-003 inside the well and not to protein-bound SRB.

Evaluation of data:

Data were evaluated as for Example 28, except that PDGF is used instead of VEGF in the formula.

Results:

To determine the appropriate concentration range for the test on inhibition of PDGF-stimulation for each compound an initial IC_{50} estimation (in the absence of additional PDGF) was done (Tables 2 and 3). 400 µM was the highest concentration (for the least toxic compound) which was tolerated by the cells. Therefore this was the maximal concentration tested in combination with PDGF.

The three compounds inhibit PDGF-stimulation of A10 rat aortic smooth muscle cells with different potencies and specificities. This is most apparent when the compounds are preincubated with the cells for 24 h before PDGF addition (Tables 4,5). The potency

of the substances is lower when the substances are added immediately before PDGF addition (especially for Compound AV-004; Tables 4,5).

In this example, the order of potency is Compound AV-004 > Compound AV-002 > Compound AV-003. At 3.2 μM, the inhibition is nearly complete with Compound AV-004, while Compound AV-002 inhibits at 5-fold higher concentrations. The effects of Compound AV-003 seem to be more nonspecific, because inhibition of the PDGF-stimulated cells only occurs at concentrations at which the unstimulated cells are also inhibited.

The differences among the activities of the compounds are clearly seen when the inhibitory IC_{50} of PDGF-stimulated cells (Tables 4,5) is compared to the inhibitory IC_{50} of unstimulated cells (Tables 2,3). In this example, there is a clear difference between the stimulated and unstimulated IC_{50} for Compound AV-002 and Compound AV-004, whereas for Compound AV-003 there is minimal to no difference. The reference compound, Doxorubicin, showed a comparable inhibition profile on stimulated and unstimulated cells, similar to Compound AV-003 (compare the IC_{50} for inhibition of PDGF-stimulated in Table 5 with the IC_{50} for inhibition of unstimulated cells in Table 3).

EXAMPLE 30: Demonstration of the Effects of Compounds in an Adjuvant Arthritis Model in Rats

This study demonstrated the effect of several of the indolinone compounds of the invention (Compound AV-002, Compound AV-003, and Compound AV-004) in an adjuvant arthritis model in rats. The adjuvant arthritis model is only one example of an animal model that can be used to test the compounds of the invention. For a review of the three most common animal models, *see* Oliver & Brahn (1996) J. Rheumatol. 23:56-60. The experiments were performed with male Wistar-Lewis rats obtained from Mollegaard Breeding Centre Ltd. The test compounds were given i.p. daily in two recommended doses for 18 days.

All three compounds showed inhibition of paw swelling, and of development of general disease symptoms. Compound AV-003 was shown to be the most effective substance, but showed an inverse dose-response at 16 mg/kg. Compound AV-004 showed a clear dose-response and was nearly as potent at 16 mg/kg as Compound AV-003 was at 8 mg/kg.

The pharmacodynamic effects of the indolinone compounds can be explained by their inhibition of receptor tyrosine kinases and resulting signal transduction to the cell nucleus which is requisite for cell proliferation. Prevention of cell proliferation inhibits neovascularization and resultant disease progression. However, the inventors do not wish to be restricted to one explanation of the data.

Test system:

The animals were divided into Control and Test groups. The Control group contained three sub-groups: healthy animals, diseased animals with arthritis, and diseased animals with arthritis treated with vehicle. The Test group contained diseased animals with arthritis treated with test compounds in vehicle. Disease was induced by injecting 0.1 mL Freud's adjuvant (=6 mg mycobacterium smegmatis suspension, per mL in heavy white paraffin oil Merck/Darmstadt) into the base of the tail.

The vehicle included PEG-400 35%, CREMOPHOR EL 25%, Benzyl alcohol 2%, Ethanol (anhydrous) 11.4%, and sterile water approx. 30%. The specific formulations for the test substances are given in Table 16 below.

TABLE 16

Formulation Excipients for Test Compounds

	Compounds AV-002 & AV-003	Compound AV-004
Excipients	(g)	(g)
PEG-400	44.80	96.00
CREMOPHOR EL	32.00	68.57
Benzyl alcohol	2.56	5.46
Ethanol (anhydrous)	14.60	31.24
Qs with water to (mL)	128.00	274.20
mg drug / mL solution	5.00	2.33

Wistar-Lewis rats (Möllegaard, Breeding Centre Ltd., EJBY, DK 4623 LI, Skensved, PO Box 28, DK) were administered 5 mL/kg of Compound AV-002 and Compound AV-003, and 10 mL/kg of Compound AV-004, i.p., adjusted with saline to the respective dose (8 mg/kg and 16 mg/kg). Rats were treated daily for days 1 thru 18. Rats

were maintained on a normal daylight schedule, given standardized food ALTROMIN® (Altrogge, Lage), and free access to water.

Observations and Measurements:

The arthritis index is calculated according to the following scheme:

1.	Ears:	0 points =	absence of nodulation in the cartilage and no adjuvant-induced reddening
		1 point =	presence of nodulation in the cartilage and adjuvant-induced reddening
2.	Nose:	0 points =	no swelling process in the connective tissue
		1 point =	severe swelling process in the connective tissue (so-called saddle-nose)
3.	Tail:	0 points =	absence of nodulation
		1 point =	presence of nodulation
4.	Front paws:	0 points =	no inflammatory reactions (= swelling)
		½ point = (per paw)	inflammation of at least one paw joint
5.	Hind paws:	0 points =	no inflammatory reactions (=swelling)
		1 point =	slight inflammation
		2 points =	moderately severe inflammatory reaction
		3 points =	severe inflammation
6.	Ballanitis:	1 point =	

The index of all animals in a group is summarized and normalized to a group size of 6 animals if necessary. Paw volume is measured plethysmographically.

Evaluation of Data:

Mean values, as well as standard deviation (SD) and standard error of the mean (SEM), were calculated from the tables of individual values in Microsoft Excel 5.0.

Results:

The three indolinone compounds, Compound AV-002, Compound AV-003, and Compound AV-004, inhibited the increase in paw volume (Fig. 1-3) and a summary of disease symptoms, expressed as a disease index is set forth in Tables 6 and 7.

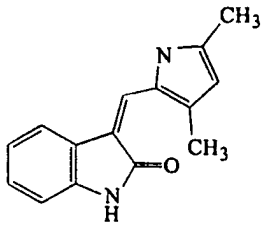
At the low dose (8 mg/kg), the order of potency was Compound AV-003 > Compound AV-004 > Compound AV-002. The inhibition of disease was nearly complete with Compound AV-003. Compound AV-004 was slightly less active. The activity of Compound AV-002 seems to be only marginal.

At the higher dose (16 mg/kg), the order of potency was Compound AV-004 > Compound AV-003 > Compound AV-002. The inhibition of disease was nearly complete with Compound AV-004. The reduction of disease symptoms with Compound AV-003 was not as marked as with the lower dose. The activity of Compound AV-002 at the higher dose was significant, but still not as pronounced as with Compound AV-003 or Compound AV-004 at the lower dose.

The course of the body weight curves (Fig. 4), indicates the best tolerability for Compound AV-003 and Compound AV-004 (body weight above diseased control + vehicle). For these compounds, the maximal tolerable dose seems to be higher than 16 mg/kg. But at least in the case of Compound AV-003, the increase in the dose from 8 to 16 mg/kg did not improve the pharmacodynamic effect.

EXAMPLE 31: Use of an Indolinone Compound to Treat Impotence.

A formulation containing the compound of formula XXIII was administered to a patient who reported significant benefit in sexual function following administration.



(XXIII)

The patient, a male cancer sufferer, had been unable to experience sexual relations because of impotence for over six months. The patient was treated with 27 mg/m^2 of the compound of formula XXIII twice per week. He was then able to experience an erection

2-3 hours following each treatment. He was able to have sexual relations by both obtaining an erection and reaching ejaculation.

EXAMPLE 32: ACTIVITY OF SOME OF THE COMPOUNDS OF THE INVENTION

The acitivity of some of the compounds of the invention was tested using the mehtods described in the above Examples. The results of these tests are set forth below in Table 17.

TABLE 17

Comp'd No.	bioPDGF IC_{50} (μM) (p-165)	bioEGF IC_{50} (μM) (p-160)	bioflk-1 IC_{50} (μM) (p-105)	bioFGF IC_{50} (μM) (p-169)	biosrc IC_{50} (μM) (p-130)	cellPDGF IC_{50} (μM) (p-114)	cellEGF IC_{50} (μM) (p-120)	PDGF-Induced BrdU Incorp. IC_{50} (μM) (p-147)	EGF-Induced BrdU Incorp. IC_{50} (μM) (p-150)	FGF-Induced BrdU Incorp. IC_{50} (μM) (p-158)
PI-001	>100	>100					>100	4.08 7.42	27.38 >50	27.16 >50
PI-002	>100						>100	9.85 >50	31.84 >50	30.79 >50
PI-003	>100	>100					>100	>50	30.95 >50	32.88 >50
PI-004	>82.8	>100					>100	2.60 3.69	27.38 >50	27.16 >50
PI-005	>100	>100					>100	>50	>50	>50
PI-006	>5.83	>100				>100	>100	>50	>50	>50
PI-007	78.7 2.38 >100	>100			>50	>100	>100	>50	>50	>50
PI-008	>100	>100		>100		>100	>100	>50	>50	>50
PI-009	>100	>100				>100	>100	>50	>50	>50
PI-010	12.9	>100		18.45		91.3	>100	1.4	>50	>50
PI-011	>100	>100		>100		>100	>100	>50	>50	>50
PI-012	>100	>100				>100	>100	>50	>50	>50
PI-013	70.63	>100				>100	>100	>50	>50	>50
PI-014	0.85 1.06	>100				5.41	>100	>50	>50	>50
PI-015	32.7	>100				>100	>100	>50	>50	>50
PI-016	72.2	>100				>100	>100	>100	>50	>50
PI-017	>100	>100		>100		>100	>100	>100	>50	>50
PI-018	>100	>100				>100	>100	>100	>50	>50
PI-019	>100	>100				>100	>100	>100	>50	>50
PI-020	94	>100				>100	>100	>50	>50	>50
PI-021	>100	>100		>100		>100	>100	>50	>50	>50
PI-022	>100	>100				>100	>100	>50	>50	>50
PI-023	0.68	11.36	0.36	0.59	2.55 4.44	15.6	>100	1.36	37.1	15.8
PI-024	1.25 5.43	>100	0.19 0.52		1.2 1.38	>100	>100			
PI-025	0.04	>100	0.08		0.74 0.93	4.25	>100			
PI-026	0.87	>100	2.72		7.52	46.88	>100			
PI-027	46.7 44.4	>100				7.69	>100			
PI-028	>100	>100				>100	>100			
PI-029	82.53 17.9	>100				7.57	>100			
PI-030	15.49	>100				2.02	>100	1.4	34.08	43.6
PI-031	95.66	>100				>100	>100	>50	>50	>50
PI-032		>100			>100	>100	>100	4.28	13.73	9.82
PI-033		>100			>100	>100	>100	13.81	19.6	15.42
PI-034		>100			>100	>100	>100			

CONCLUSION

One skilled in the art would readily appreciate that the present invention is well adapted to carry out the objects and obtain the ends and advantages mentioned, as well as those inherent herein. The molecular complexes and the methods, procedures, treatments, molecules, specific compounds described herein are presently representative of preferred embodiments, are exemplary, and are not intended as limitations on the scope of the invention. Changes therein and other uses will occur to those skilled in the art which are encompassed within the spirit of the invention are defined by the scope of the claims.

It will be readily apparent to one skilled in the art that varying substitutions and modifications may be made to the invention disclosed herein without departing from the scope and spirit of the invention.

All patents and publications mentioned in the specification are indicative of the levels of those skilled in the art to which the invention pertains. All patents and publications are herein incorporated by reference to the same extent as if each individual publication was specifically and individually indicated to be incorporated by reference.

The invention illustratively described herein suitably may be practiced in the absence of any element or elements, limitation or limitations which is not specifically disclosed herein. Thus, for example, in each instance herein any of the terms "comprising", "consisting essentially of" and "consisting of" may be replaced with either of the other two terms. The terms and expressions which have been employed are used as terms of description and not of limitation, and there is no intention that in the use of such terms and expressions of excluding any equivalents of the features shown and described or portions thereof, but it is recognized that various modifications are possible within the scope of the invention claimed. Thus, it should be understood that although the present invention has been specifically disclosed by preferred embodiments and optional features, modification and variation of the concepts herein disclosed may be resorted to by those skilled in the art, and that such modifications and variations are considered to be within the scope of this invention as defined by the appended claims.

In addition, where features or aspects of the invention are described in terms of Markush groups, those skilled in the art will recognize that the invention is also thereby described in terms of any individual member or subgroup of members of the Markush

group. For example, if X is described as selected from the group consisting of bromine, chlorine, and iodine, claims for X being bromine and claims for X being bromine and chlorine are fully described.

The invention has been described broadly and generically herein. Each of the narrower species and subgeneric groupings falling within the generic disclosure also form part of the invention. This includes the generic description of the invention with a proviso or negative limitation removing any subject matter from the genus, regardless of whether or not the excised material is specifically recited herein.

Other embodiments are within the following claims.

Table 1: Summary of IC_{50} determination of test substances of HUVEC and inhibition of VEGF-stimulation by test substances without preincubation

group	Compound AV-002 in µM										IC50in µM
	control	400	80	16	3.2	0.64	0.128	0.0256	0.00512	0.0010	
control	0.568	0.062	0.094	0.139	0.457	0.531	0.551	0.546	0.543	0.576	8.7
stand deviation +/-	0.017	0.005	0.004	0.009	0.027	0.017	0.011	0.004	0.023	0.015	
VEGF [10ng/m]	0.849	0.062	0.108	0.168	0.459	0.571	0.749	0.791	0.823	0.815	
stand deviation +/-	0.013	0.002	0.013	0.024	0.021	0.012	0.018	0.019	0.018	0.023	
inhibition of stimulation in %	0.0	100.1	95.1	89.8	99.1	51.9	31.8	15.9	3.8	18.1	0.34

group	Compound AV-003 in µM											IC50in µM
	control	400	80	16	3.2	0.64	0.128	0.0256	0.00512	0.0010	0.0002	
control	0.673	0.234	0.243	0.415	0.492	0.505	0.493	0.514	0.569	0.603	0.618	37.4
stand deviation +/-	0.013	0.043	0.008	0.007	0.012	0.007	0.004	0.019	0.018	0.007	0.016	
VEGF [10ng/m]	0.927	0.238	0.258	0.462	0.563	0.589	0.538	0.827	0.876	0.881	0.899	
stand devation +/-	0.034	0.032	0.005	0.007	0.017	0.014	0.009	0.008	0.031	0.029	0.014	
inhibition of stimulation %	0.0	98.3	90.2	81.3	76.2	74.7	42.9	-22.9	-20.9	-9.3	-10.6	0.28

Table 1 (cont'd)

group	Compound AV-004 in µM											IC50in µM
	control	400	80	16	3.2	0.64	0.128	0.0256	0.00512	0.0010	0.0002	
control	0.025	0.344	0.112	0.198	0.335	0.444	0.490	0.492	0.482	0.503	0.502	2.5
stand deviation +/-	0.018	0.024	0.004	0.008	0.012	0.017	0.018	0.006	0.013	0.012	0.008	
VEGF [10ng/m]	0.829	0.366	0.120	0.254	0.558	0.687	0.717	0.734	0.723	0.744	0.740	
stand devation +/-	0.009	0.014	0.012	0.007	0.014	0.012	0.014	0.013	0.016	0.013	0.014	
inhibition of stimulation %	0.0	89.5	96.2	72.3	-9.8	-19.2	-11.7	-19.3	-18.3	-18.3	-17.1	14.1

group	doxorubicin in µM											IC50in µM
	control	400	80	16	3.2	0.64	0.128	0.0256	0.00512	0.0010	0.0002	
control	0.598	0.924	0.117	0.059	0.046	0.763	0.397	0.447	0.534	0.578	0.576	0.2
stand deviation +/-	0.025	0.052	0.002	0.003	0.003	0.008	0.013	0.017	0.021	0.015	0.024	
VEGF [10ng/m]	0.796	0.946	0.123	0.062	0.046	0.221	0.667	0.642	0.723	0.771	0.779	
stand devation +/-	0.017	0.071	0.001	0.004	0.002	0.015	0.006	0.010	0.007	0.011	0.006	
inhibition of stimulation %	0.0	88.6	96.8	98.2	100.3	70.5	19.0	1.3	4.3	2.7	-2.7	0.35

Table 2: Summary of IC_{50} determination of test substances on A10 cells without preincubation (without PDGF)

% control

substance	concentration in µM									IC50in µM
	0	400	80	16	3.2	0.64	0.128	0.0256	0.00512	
Compd AV-002	100	82	75	88	93	97	94	95	94	>400
Compd AV-003	100	74	31	82	101	101	102	107	104	46.1
Compd AV-004	100	63	80	93	93	93	92	91	90	>400

Table 3: Summary of IC_{50} determination of reference and test substances on A10 cells with preincubation (without PDGF)

% control

substance	concentration in µM									IC50in µM
	0	400	80	16	3.2	0.64	0.128	0.0256	0.00512	
Compd AV-002	100	55	79	89	97	98	95	96	103	>400
Compd AV-003	100	74	21	47	92	94	96	97	95	17.5
Compd AV-004	100	32	67	87	87	88	97	85	84	172

% control

substance	concentration in µM										
	0	100	20	4	0.8	0.16	0.032	0.0064	0.00128	0.000256	
doxorubicin	100	12	10	8	18	52	57	73	82	883	IC50 0.064 µM

Table 4: Summary of inhibition of PDGF-stimulation by test substances without preincubation

extinction

substance	Compound AV-002 in µM									
	0	400	80	16	3.2	0.64	0.128	0.0256	0.00512	
control	0.645	0.388	0.485	0.568	0.601	0.628	0.606	0.611	0.607	
stand deviation +/-	0.010	0.041	0.014	0.007	0.013	0.012	0.012	0.007	0.007	
PDGF 0.1 µg/ml	1.245	0.642	0.539	0.677	1.103	1.256	1.129	1.131	1.116	
stand deviation +/-	0.028	0.141	0.012	0.018	0.048	0.038	0.020	0.025	0.029	
inhibition of stimulation in %	0.0	59.3	91.0	82.0	16.2	-4.7	12.8	13.3	15.2	IC507.5 µM

substance	Compound AV-003 in µM									
	0	400	80	16	3.2	0.64	0.128	0.0256	0.00512	
control	0.694	0.514	0.216	0.572	0.699	0.700	0.706	0.741	0.722	
stand deviation +/-	0.029	0.032	0.012	0.034	0.044	0.020	0.031	0.026	0.038	
PDGF 0.1 µg/ml	1.150	0.485	0.143	0.902	1.079	1.068	1.090	1.083	1.105	
stand deviation +/-	0.011	0.027	0.010	0.010	0.030	0.017	0.017	0.012	0.008	
inhibition of stimulation in %	0.0	106.4	116.0	27.5	16.7	19.2	15.8	24.9	16.0	IC5020.1 µM

Table 4 (Cont'd)

substance	Compound AV-004 in µM									
	0	400	80	16	3.2	0.64	0.128	0.0256	0.00512	
control	0.698	0.442	0.559	0.650	0.650	0.647	0.640	0.634	0.627	
stand deviation +/-	0.034	0.020	0.019	0.025	0.021	0.028	0.018	0.015	0.016	
PDGF 0.1 µg/ml	1.208	0.500	0.726	0.888	0.892	1.052	1.184	1.208	1.128	
stand deviation +/-	0.036	0.044	0.022	0.026	0.025	0.054	0.050	0.040	0.037	
inhibition of stimulation in %	0.0	88.8	67.2	53.4	52.5	20.7	-6.7	-12.4	1.8	IC507.4 µM

Table 5: Summary of inhibition of PDGF-stimulation by reference and test substances with preincubation

substance	Compound AV-002 in µM									
	0	400	80	16	3.2	0.64	0.128	0.0256	0.00512	
control	0.875	0.484	0.691	0.781	0.852	0.855	0.834	0.857	0.905	
stand deviation +/-	0.048	0.037	0.042	0.037	0.053	0.038	0.041	0.058	0.075	
PDGF 0.1 µg/ml	1.587	0.597	0.792	0.854	1.279	1.581	1.528	1.590	1.573	
stand deviation +/-	0.076	0.078	0.075	0.065	0.060	0.079	0.065	0.086	0.088	
inhibition of stimulation in %	0.0	84.2	85.8	75.7	40.1	-2.1	2.4	-3.0	6.1	IC504.6 µM

substance	Compound AV-003 in µM									
	0	400	80	16	3.2	0.64	0.128	0.0256	0.00512	
control	0.777	0.575	0.162	0.382	0.713	0.728	0.746	0.754	0.741	
stand deviation +/-	0.010	0.055	0.009	0.008	0.005	0.013	0.015	0.015	0.010	
PDGF 0.1 µg/ml	1.452	0.613	0.211	0.736	1.355	1.382	1.390	1.401	1.403	
stand deviation +/-	0.030	0.027	0.025	0.067	0.010	0.014	0.009	0.014	0.018	
inhibition of stimulation in %	0.0	94.3	92.9	44.6	5.0	3.1	4.7	4.1	2.0	IC5018.2 µM

Table 5 (Cont'd)

substance	Compound AV-004 in µM									
	0	400	80	16	3.2	0.64	0.128	0.0256	0.00512	
control	0.763	0.241	0.513	0.666	0.663	0.572	0.663	0.658	0.640	
stand deviation +/-	0.054	0.028	0.020	0.017	0.023	0.015	0.015	0.040	0.012	
PDGF 0.1 µg/ml	1.384	0.298	0.696	0.802	0.824	0.942	1.209	1.333	1.334	
stand deviation +/-	0.028	0.025	0.022	0.031	0.018	0.044	0.030	0.041	0.025	
inhibition of stimulation in %	0.0	90.8	70.6	78.2	74.1	56.5	12.0	-8.8	-11.8	IC500.97 µM

extinction

substance	Doxorubicin in µM										
	0	100	20	4	0.8	0.16	0.032	0.0064	0.00128	0.000256	
control	0.635	0.078	0.063	0.052	0.116	0.331	0.359	0.461	0.520	0.557	
stand deviation +/-	0.021	0.009	0.012	0.011	0.009	0.007	0.006	0.007	0.011	0.019	
PDGF 0.1 µg/ml	1.467	0.094	0.071	0.065	0.289	0.609	0.653	0.934	1.164	1.296	
stand deviation +/-	0.009	0.011	0.010	0.009	0.016	0.017	0.007	0.013	0.011	0.038	
inhibition of stimulation in %	0.0	98.0	99.1	98.5	79.3	66.6	64.6	43.2	22.7	11.1	IC500.016

Table 6: Summary of effects of test compounds in the adjuvant arthritis rat model compared to arthritis control + vehicle (772-22)

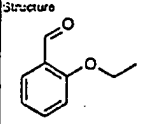
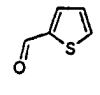
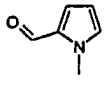
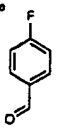
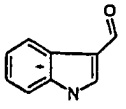
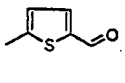
Adjuvant Arthritis		Test No: 186	% Change Day 18		Drug Treatment: Day: 1-18 i.p.	
No	Compound	Dose mg/kg	Paw Volume 1)	Weight 2)	Index 1)	
1	Healthy Control			21		
2	Arthritis Control		37	10	44	(untreated)
3	Arthritis Control +	Vehicle		13		(5 ml/kg)
4	Compound AV-002	8	17	12	20	--
5	Compound AV-002	16	-28	17	-35	--
6	Compound AV-003	8	-59	25	-79	--
7	Compound AV-003	16	-33	16	-39	--
8	Compound AV-004	8	-44	15	-61	(10 ml/kg)
9	Compound AV-004	16	-57	16	-64	--

1) Compared to Arthritis - Control (Vehicle)
2) Bodyweight gain compared to day one

Table 7: Summary of effects of test compounds on the index in adjuvant arthritis rats compared to arthritis control + vehicle (772-22)

Adjuvant Arthritis			Index
	Test No.: 186,		Day: 18
	Index	_%	
Arthritis Control	37.75	44	
Arthritis Control + 772-22	26.3		
Compound AV-002, 8 mg/kg i.p.	31.5	20	
Compound AV-002, 16 mg/kg i.p.	17.0	-35	
Compound AV-003, 8 mg/kg i.p.	5.5	-79	
Compound AV-003, 16 mg/kg i.p.	16.0	-39	
Compound AV-004, 8 mg/kg i.p.	10.2	-61	
Compound AV-004, 16 mg/kg i.p.	9.5	-64	

TABLE 8

Structure	Structure	Structure	Structure	Structure	Structure	Structure
						
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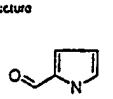
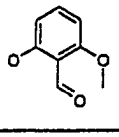
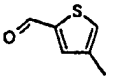
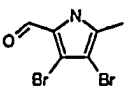
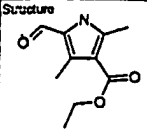
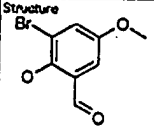
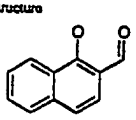
Structure	Structure	Structure	Structure	Structure	Structure	Structure
						
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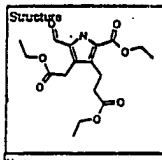
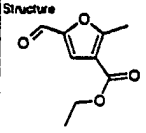
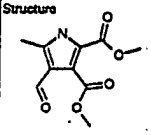
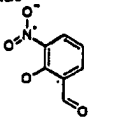
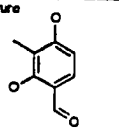
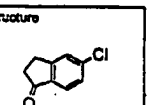
Structure	Structure	Structure	Structure	Structure	Structure	Structure
						
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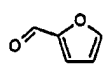
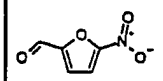
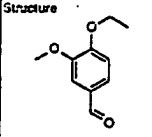
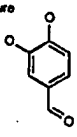
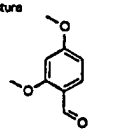
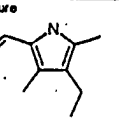
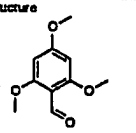
Structure	Structure	Structure	Structure	Structure	Structure	Structure
						
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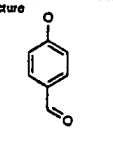
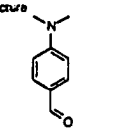
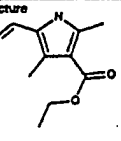
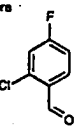
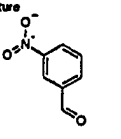
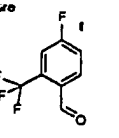
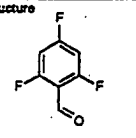
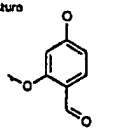
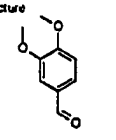
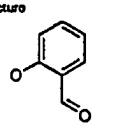
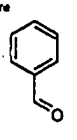
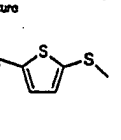
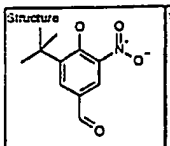
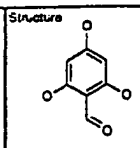
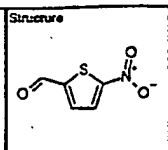
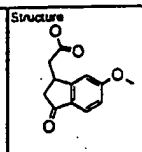
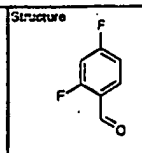
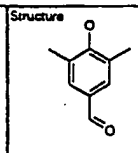
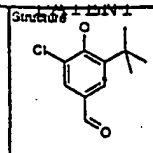
Structure	Structure	Structure	Structure	Structure	Structure	Structure
						
Name CHO-028	Name CHO-029	Name CHO-030	Name CHO-031	Name CHO-032	Name CHO-033	Name CHO-034
Structure	Structure	Structure	Structure	Structure	Structure	Structure
						
Name CHO-035	Name CHO-036	Name CHO-037	Name CHO-038	Name CHO-039	Name CHO-040	Name CHO-041
Structure	Structure	Structure	Structure	Structure	Structure	Structure
Name CHO-042	Name CHO-043	Name CHO-044	Name CHO-045	Name CHO-046	Name CHO-047	Name CHO-048
Structure	Structure	Structure	Structure	Structure	Structure	Structure
Name CHO-049	Name CHO-050	Name CHO-051	Name CHO-052	Name CHO-053	Name CHO-054	Name CHO-055

TABLE 8 (continued)

Structure	Structure	Structure	Structure	Structure	Structure	Structure
						
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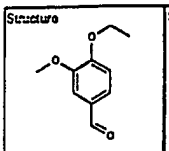
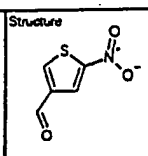
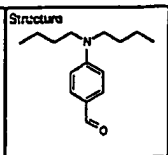
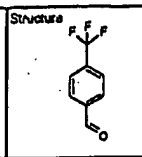
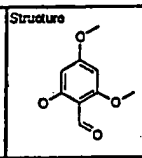
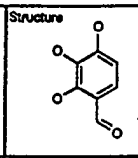
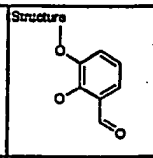
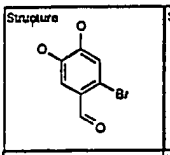
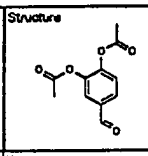
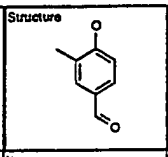
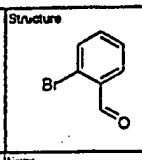
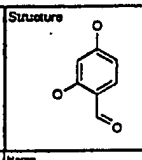
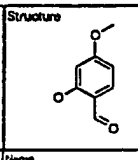
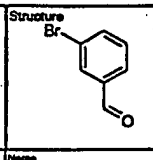
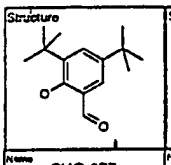
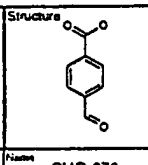
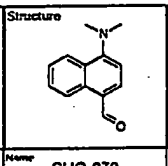
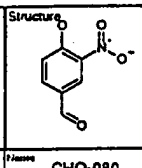
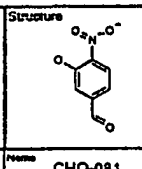
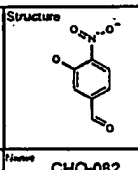
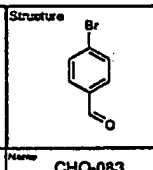
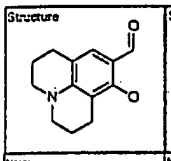
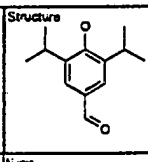
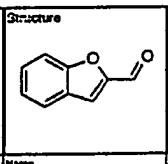
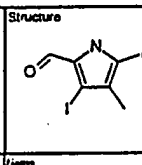
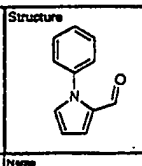
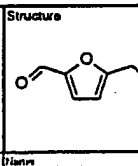
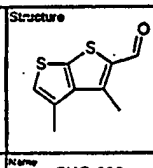
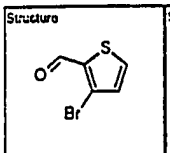
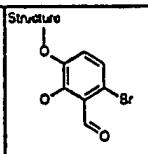
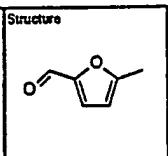
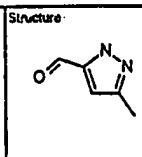
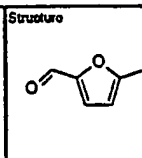
Structure	Structure	Structure	Structure	Structure	Structure	Structure
						
Name CHO-063	Name CHO-064	Name CHO-065	Name CHO-066	Name CHO-067	Name CHO-068	Name CHO-069
Structure	Structure	Structure	Structure	Structure	Structure	Structure
						
Name CHO-070	Name CHO-071	Name CHO-072	Name CHO-073	Name CHO-074	Name CHO-075	Name CHO-076
Structure	Structure	Structure	Structure	Structure	Structure	Structure
						
Name CHO-077	Name CHO-078	Name CHO-079	Name CHO-080	Name CHO-081	Name CHO-082	Name CHO-083
Structure	Structure	Structure	Structure	Structure	Structure	Structure
						
Name CHO-084	Name CHO-085	Name CHO-086	Name CHO-087	Name CHO-088	Name CHO-089	Name CHO-090
Structure	Structure	Structure	Structure	Structure	Structure	Structure
						
Name CHO-091	Name CHO-092	Name CHO-093	Name CHO-094	Name CHO-095	Name CHO-096	Name CHO-097
Structure	Structure	Structure	Structure	Structure	Structure	Structure
Name CHO-098	Name CHO-099	Name CHO-100	Name CHO-101	Name CHO-102	Name CHO-103	Name CHO-104
Structure	Structure	Structure	Structure	Structure	Structure	Structure
Name CHO-105	Name CHO-106	Name CHO-107	Name CHO-108	Name CHO-109	Name CHO-110	Name CHO-111

TABLE 8 (continued)

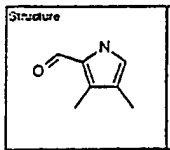
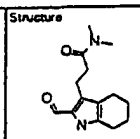
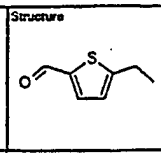
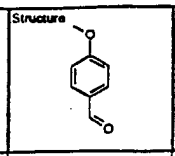
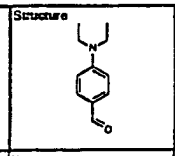
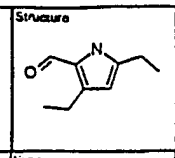
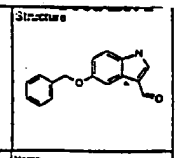
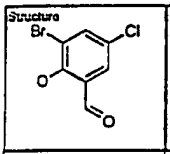
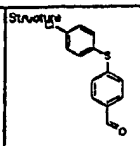
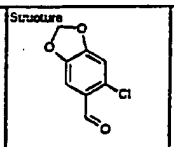
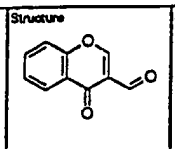
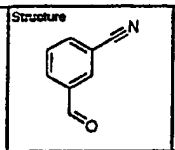
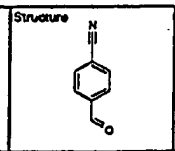
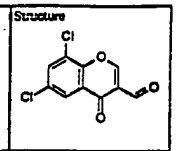
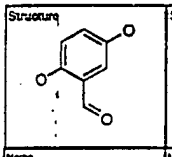
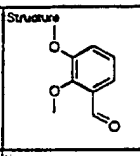
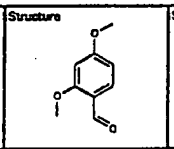
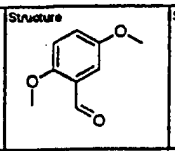
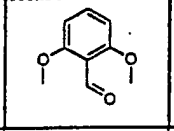
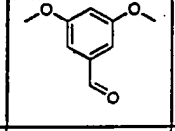
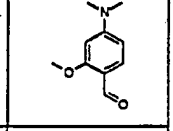
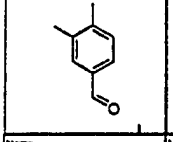
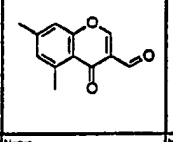
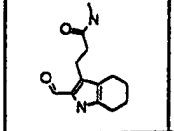
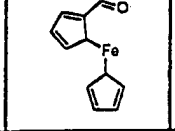
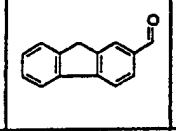
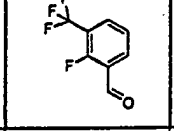
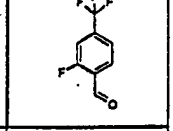
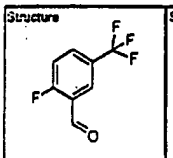
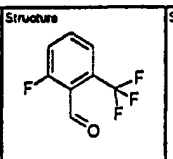
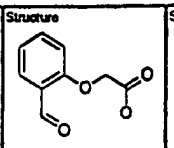
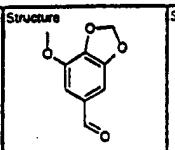
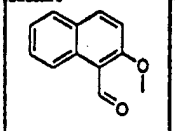
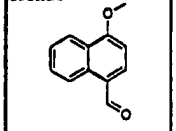
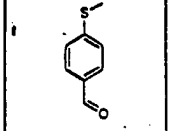
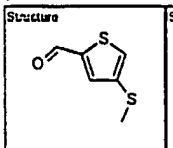
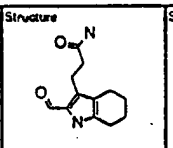
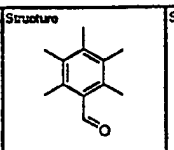
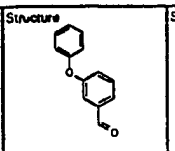
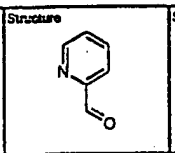
Structure	Structure	Structure	Structure	Structure	Structure	Structure
						
Name CHO-112	Name CHO-113	Name CHO-114	Name CHO-115	Name CHO-116	Name CHO-117	Name CHO-118
Structure	Structure	Structure	Structure	Structure	Structure	Structure
						
Name CHO-119	Name CHO-120	Name CHO-121	Name CHO-122	Name CHO-123	Name CHO-124	Name CHO-125
Structure	Structure	Structure	Structure	Structure	Structure	Structure
						
Name CHO-126	Name CHO-127	Name CHO-128	Name CHO-129	Name CHO-130	Name CHO-131	Name CHO-132
Structure	Structure	Structure	Structure	Structure	Structure	Structure
						
Name CHO-133	Name CHO-134	Name CHO-135	Name CHO-136	Name CHO-137	Name CHO-138	Name CHO-139
Structure	Structure	Structure	Structure	Structure	Structure	Structure
						
Name CHO-140	Name CHO-141	Name CHO-142	Name CHO-143	Name CHO-144	Name CHO-145	Name CHO-146
Structure	Structure	Structure	Structure	Structure	Structure	Structure
						
Name CHO-147	Name CHO-148	Name CHO-149	Name CHO-150	Name CHO-151	Name CHO-152	Name CHO-153
Structure	Structure	Structure	Structure	Structure	Structure	Structure
Name CHO-154	Name CHO-155	Name CHO-156	Name CHO-157	Name CHO-158	Name CHO-159	Name CHO-160
Structure	Structure	Structure	Structure	Structure	Structure	Structure
Name CHO-161	Name CHO-162	Name CHO-163	Name CHO-164	Name CHO-165	Name CHO-166	Name CHO-167

TABLE 8 (continued)

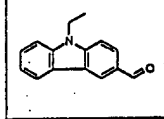
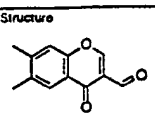
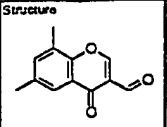
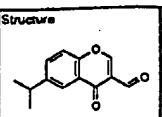
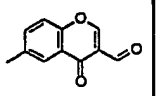
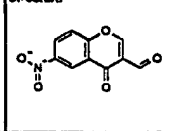
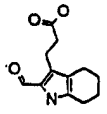
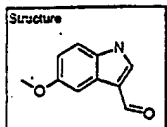
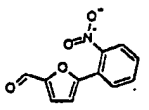
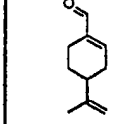
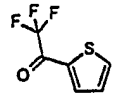
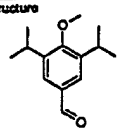
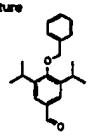
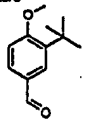
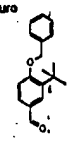
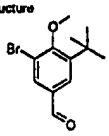
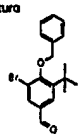
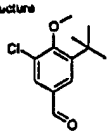
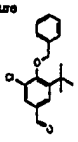
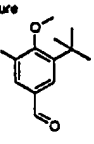
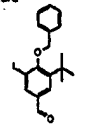
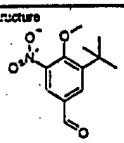
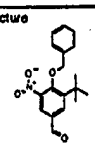
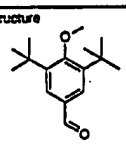
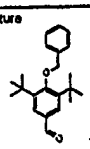
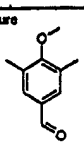
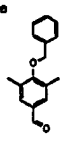
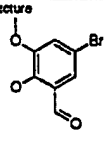
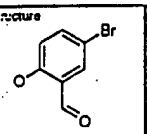
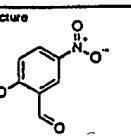
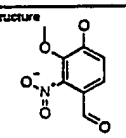
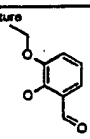
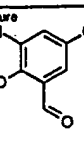
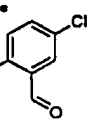
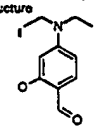
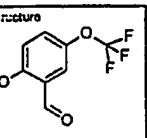
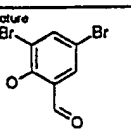
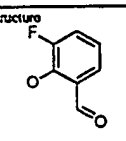
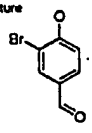
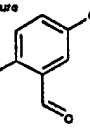
Structure	Structure	Structure	Structure	Structure	Structure	Structure
						
Name CHO-168	Name CHO-169	Name CHO-170	Name CHO-171	Name CHO-172	Name CHO-173	Name CHO-174
Structure	Structure	Structure	Structure	Structure	Structure	Structure
						
Name CHO-175	Name CHO-177	Name CHO-178	Name CHO-179	Name CHO-180	Name CHO-181	Name CHO-182
Structure	Structure	Structure	Structure	Structure	Structure	Structure
						
Name CHO-183	Name CHO-184	Name CHO-185	Name CHO-186	Name CHO-187	Name CHO-188	Name CHO-189
Structure	Structure	Structure	Structure	Structure	Structure	Structure
						
Name CHO-190	Name CHO-191	Name CHO-192	Name CHO-193	Name CHO-194	Name CHO-195	Name CHO-196
Structure	Structure	Structure	Structure	Structure	Structure	Structure
						
Name CHO-197	Name CHO-198	Name CHO-199	Name CHO-200	Name CHO-201	Name CHO-202	Name CHO-203
Structure	Structure	Structure	Structure	Structure	Structure	Structure
						
Name CHO-204	Name CHO-205	Name CHO-206	Name CHO-207	Name CHO-208	Name CHO-209	Name CHO-210
Structure	Structure	Structure	Structure	Structure	Structure	Structure
Name CHO-211	Name CHO-215	Name CHO-216	Name CHO-217	Name CHO-223	Name CHO-224	Name CHO-225
Structure	Structure	Structure	Structure	Structure	Structure	Structure
Name CHO-229	Name CHO-232	Name CHO-237	Name CHO-238	Name CHO-239	Name CHO-240	Name CHO-241

TABLE 8 (continued)

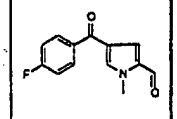
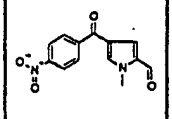
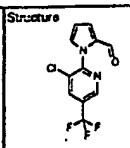
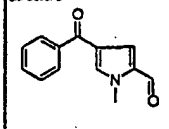
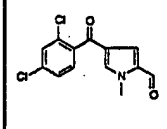
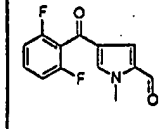
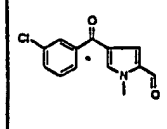
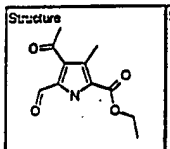
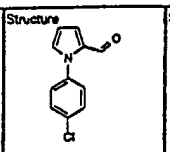
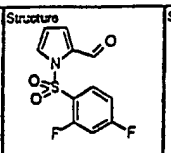
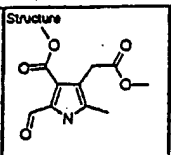
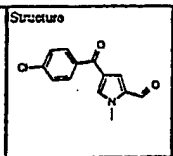
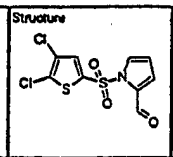
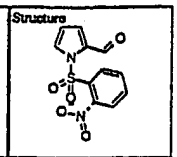
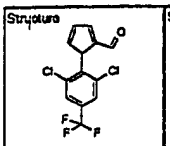
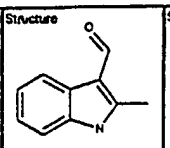
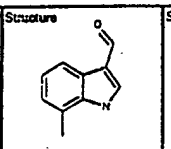
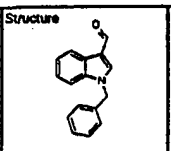
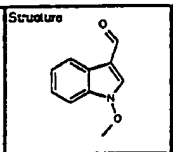
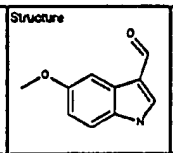
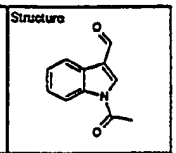
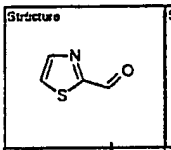
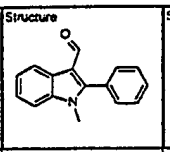
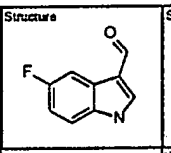
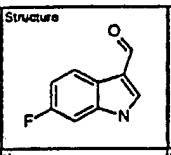
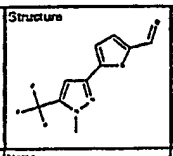
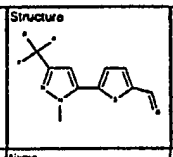
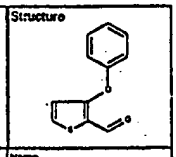
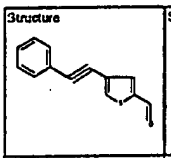
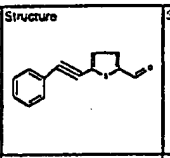
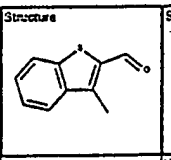
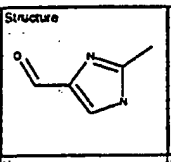
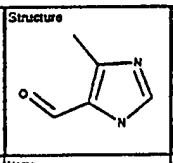
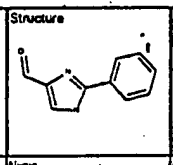
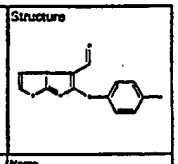
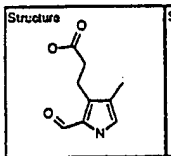
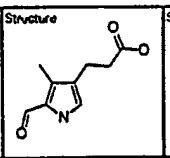
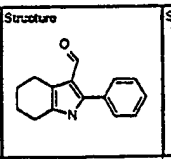
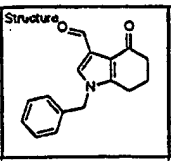
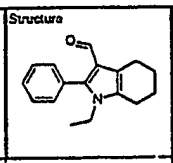
Structure	Structure	Structure	Structure	Structure	Structure	Structure
						
Name CHO-242	Name CHO-243	Name CHO-244	Name CHO-245	Name CHO-246	Name CHO-247	Name CHO-248
Structure	Structure	Structure	Structure	Structure	Structure	Structure
						
Name CHO-249	Name CHO-250	Name CHO-251	Name CHO-252	Name CHO-253	Name CHO-254	Name CHO-255
Structure	Structure	Structure	Structure	Structure	Structure	Structure
						
Name CHO-256	Name CHO-257	Name CHO-258	Name CHO-259	Name CHO-260	Name CHO-261	Name CHO-262
Structure	Structure	Structure	Structure	Structure	Structure	Structure
						
Name CHO-263	Name CHO-264	Name CHO-265	Name CHO-266	Name CHO-267	Name CHO-268	Name CHO-269
Structure	Structure	Structure	Structure	Structure	Structure	Structure
						
Name CHO-270	Name CHO-271	Name CHO-272	Name CHO-273	Name CHO-274	Name CHO-275	Name CHO-276
Structure	Structure	Structure	Structure	Structure	Structure	Structure
						
Name CHO-277	Name CHO-278	Name CHO-279	Name CHO-280	Name CHO-281	Name CHO-282	Name CHO-283
Structure	Structure	Structure	Structure	Structure	Structure	Structure
Name CHO-284	Name CHO-285	Name CHO-286	Name CHO-287	Name CHO-288	Name CHO-289	Name CHO-290
Structure	Structure	Structure	Structure	Structure	Structure	Structure
Name CHO-291	Name CHO-292	Name CHO-293	Name CHO-294	Name CHO-295	Name CHO-296	Name CHO-297

TABLE 8 (continued)

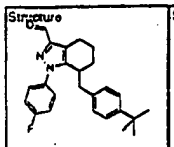
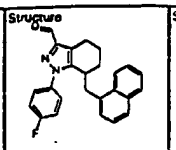
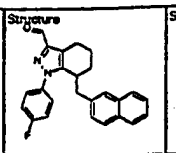
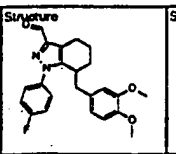
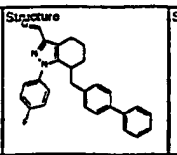
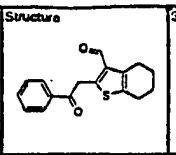
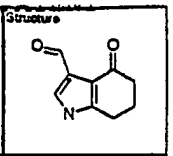
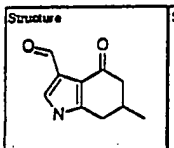
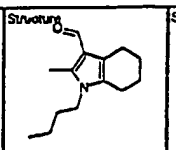
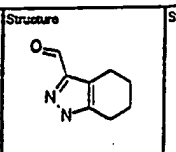
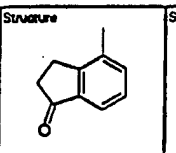
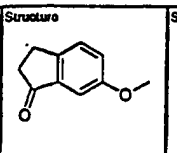
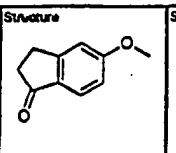
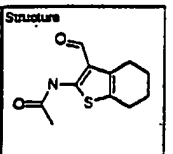
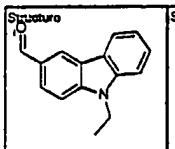
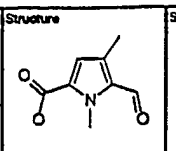
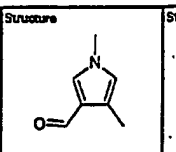
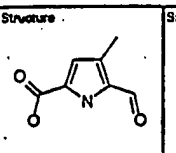
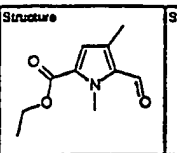
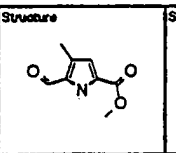
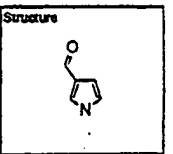
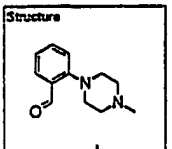
Structure	Structure	Structure	Structure	Structure	Structure	Structure
						
Name CHO-298	Name CHO-299	Name CHO-300	Name CHO-301	Name CHO-302	Name CHO-303	Name CHO-304
Structure	Structure	Structure	Structure	Structure	Structure	Structure
						
Name CHO-305	Name CHO-306	Name CHO-307	Name CHO-308	Name CHO-309	Name CHO-310	Name CHO-311
Structure	Structure	Structure	Structure	Structure	Structure	Structure
						
Name CHO-312	Name CHO-313	Name CHO-314	Name CHO-315	Name CHO-316	Name CHO-317	Name CHO-318
Structure						
						
Name CHO-319						

TABLE 9

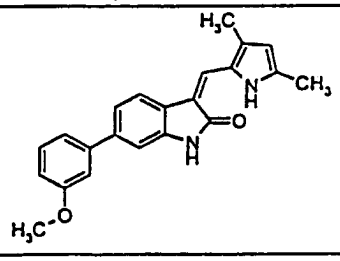
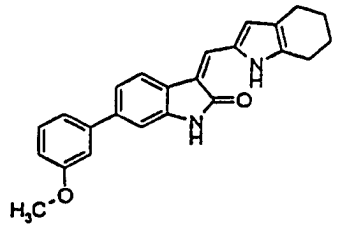
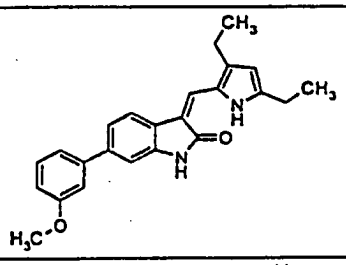
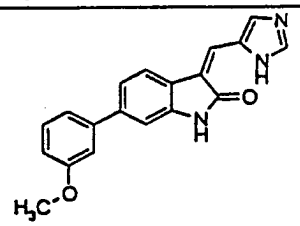
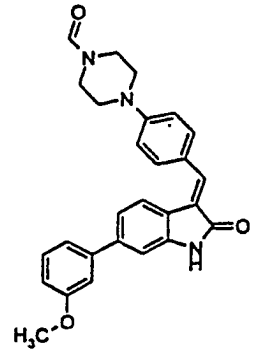
Compound Nunber	Structure
PI-00$_1$	
PI-002	
PI-003	
PI-004	
PI-005	

TABLE 9 (continued)

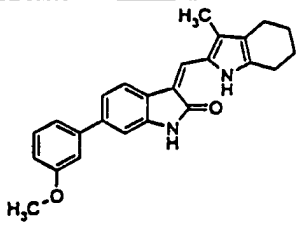
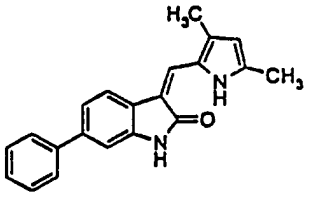
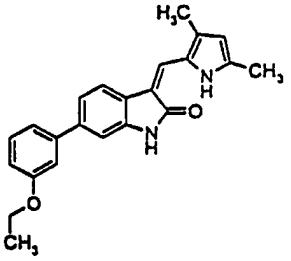
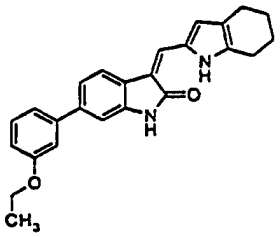
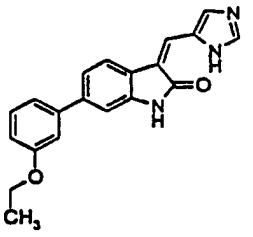
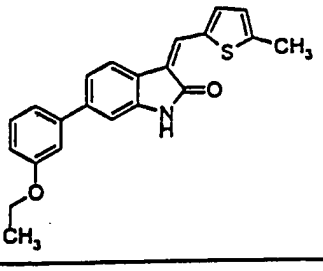
PI-006	
PI-007	
PI-008	
PI-009	
PI-010	
PI-011	

TABLE 9 (continued)

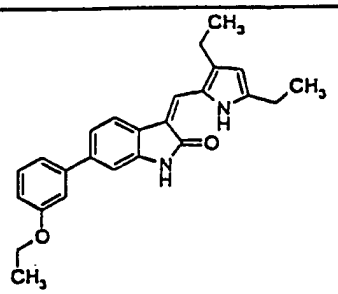
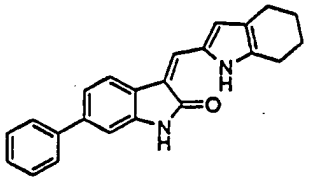
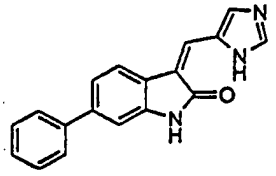
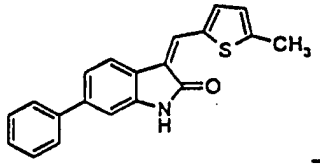
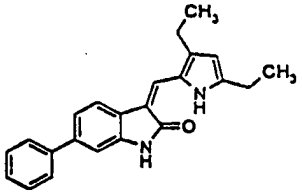
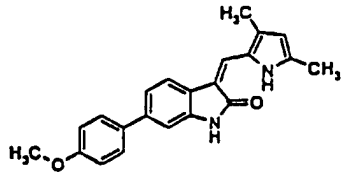
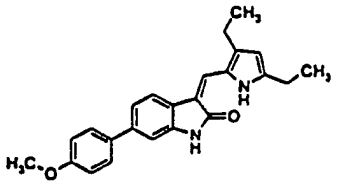
PI-012	
PI-013	
PI-014	
PI-015	
PI-016	
PI-017	
PI-018	

TABLE 9 (continued)

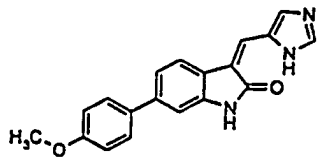
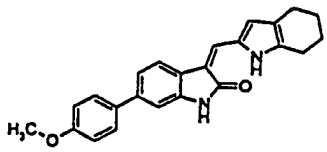
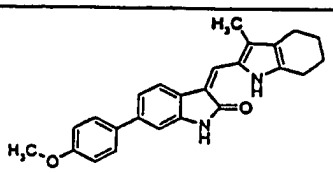
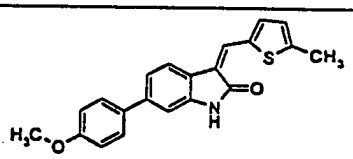
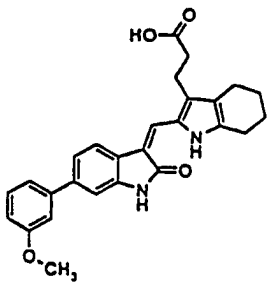
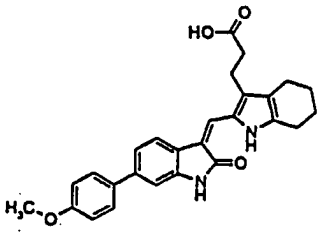
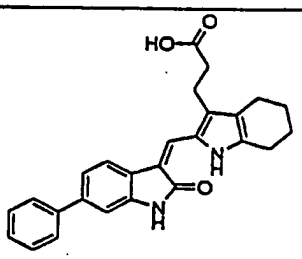
PI-019	
PI-020	
PI-021	
PI-022	
PI-023	
PI-024	
PI-025	

TABLE 9 (continued)

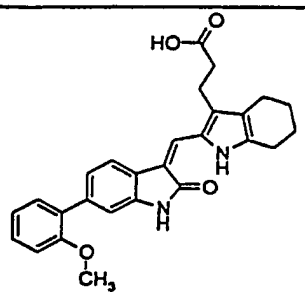
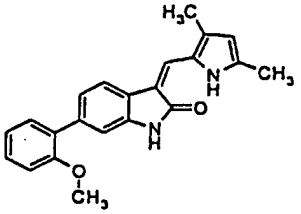
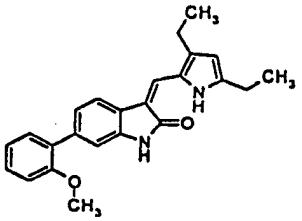
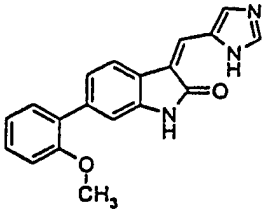
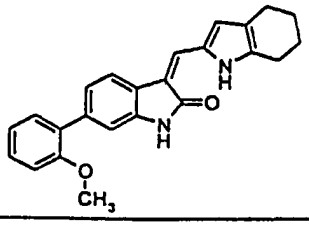
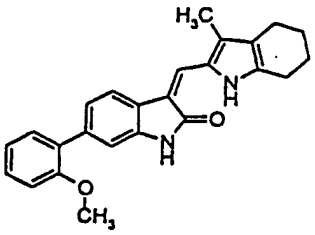
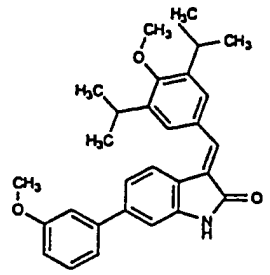
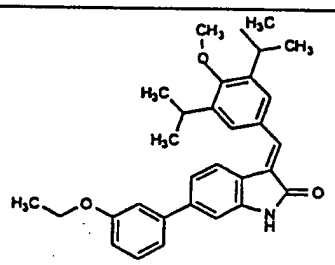
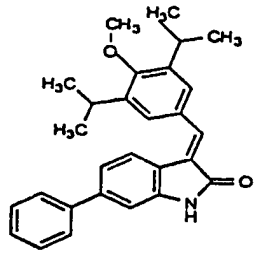
PI-026	
PI-027	
PI-028	
PI-029	
PI-030	
PI-031	

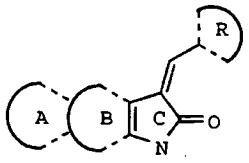
TABLE 9 (continued) PATENT

PI-032	
PI-033	
PI-034	

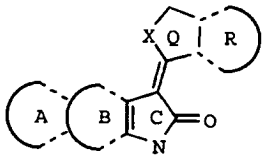
What is Claimed is:

CLAIMS

1. A tricyclic-based indolinone compound having a structure set forth in formula I or II:



(I)



(II)

wherein

(a) ring A and ring B share one common bond;

(b) ring B and ring C share one common bond;

(c) ring A, ring B, and ring R are independently selected from the group consisting of an aromatic ring, a heteroaromatic ring, an aliphatic ring, a heteroaliphatic ring, and a fused aromatic or aliphatic ring system, wherein said heteroaromatic ring and heteroaliphatic ring each independently contain 0, 1, 2, or 3 heteroatoms independently selected from the group consisting of nitrogen, oxygen, and sulfur;

(d) ring A, ring B, ring Q, and ring R are each independently and optionally substituted with one, two, or three substituents independently selected from the group consisting of (i) alkyl; (ii) an aromatic or heteroaromatic ring; (iii) an aliphatic or heteroaliphatic ring; (iv) an amine; (v) a nitro of formula $-NO_2$; (vi) a halogen or trihalomethyl; (vii) a ketone; (viii) a carboxylic acid or ester; (ix) an alcohol or an alkoxyalkyl moiety; (x) an amide; (xi) a sulfonamide; (xii) an aldehyde; (xiii) a sulfone; (xiv) a thiol or a thioether; and (xv) a heavy metal, substituted with a five-membered or six-membered aromatic or heteroaromatic ring moieties, wherein said ring is optionally substituted with one or more substituents independently selected from the group consisting of alkyl, halogen, trihalomethyl, carboxylate, amino, nitro, and ester; and

(e) X is selected from the group consisting of CH and oxygen.

2. The compound of claim 1, wherein ring A is selected from the group consisting of a 5-membered ring, a 6-membered ring, a 7-membered ring, and an 8-membered ring.

3. The compound of claim 1, wherein ring B is selected from the group consisting of a 5-membered ring, a 6-membered ring, a 7-membered ring, and an 8-membered ring.

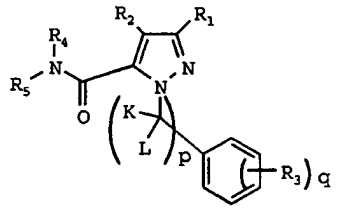
4. The compound of claim 1, wherein ring R is selected from the group consisting of a 5-membered ring, a 6-membered ring, a 7-membered ring, an 8-membered ring, and a bicyclic or tricyclic fused ring system.

5. The compound of claim 4, wherein R is a bicyclic fused ring system comprising 8, 9, 10, or 13 atoms in the ring backbone.

6. The compound of claim 4, wherein R is an optionally substituted ferrocene.

7. The compound of claim 4, wherein R is derived from an aldehyde, ketone, or lactone selected from the group consisting of the compounds set forth in Table 8.

8. A pyrazolylamide-based compound having a structure set forth in formula X:



(X)

wherein

(a) R_1 and R_2 are independently selected from the group consisting of (i) hydrogen; (ii) alkyl; (iii) an aromatic or heteroaromatic ring; (iv) an aliphatic or heteroaliphatic ring; (v) an amine; (vi) a nitro of formula $-NO_2$; (vii) a halogen; (viii) a

ketone; (ix) a carboxylic acid or ester; (x) an alcohol or an alkoxyalkyl moiety; (xi) an amide; (xii) a sulfonamide; (xiii) an alkoxyalkoxy; and (xiv) a sulfone; and

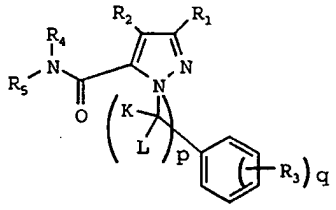
(b) R_4 and R_5 are each independently selected from the group consisting of (i) hydrogen; (ii) alkyl; (iii) an aromatic ring; (iv) a heteroaromatic ring; (v) an aliphatic or heteroaliphatic; (vi) an amine; (vii) a ketone (viii) a carboxylic acid or ester; (ix) an alcohol or an alkoxyalkyl moiety; (x) an amide; (xi) a sulfonamide; (xii) a sulfone; and (xiii) an alkoxyalkoxy; and

(c) R_3 is selected from the group consisting of (i) hydrogen; (ii) alkyl; (iii) an aromatic or heteroaromatic ring; (iv) an aliphatic or heteroaliphatic ring; (v) a halogen or trihalomethyl; (vi) an amine; (vii) an amide; (viii) an alcohol or an alkoxyalkyl moiety; (ix) a carboxylic acid or ester; (x) a cyano of formula -CN; and (xi) a sulfonamide;

(d) p and q are each independently 0, 1, 2, or 3; and

(e) K and L are each independently selected from the gruop consisting of (i) hydrogen; (ii) alkyl; and (iii) K and L taken together form a three-membered, four-membered, five-membered, or six-membered aliphatic ring.

9. A pyrazolylamide-based compound having a structure set forth in formula X:



(X)

wherein

(a) R_1, R_4, and R_5 are independently selected from the group consisting of (i) hydrogen; (ii) alkyl; (iii) an aromatic or heteroaromatic ring; (iv) an aliphatic or heteroaliphatic ring; (v) an amine; (vi) a nitro of formula $-NO_2$; (vii) a halogen; (viii) a ketone; (ix) a carboxylic acid or ester; (x) an alcohol or an alkoxyalkyl moiety; (xi) an amide; (xii) a sulfonamide; (xiii) an alkoxyalkoxy; and (xiv) a sulfone; and

wherein at least one of R_4 and R_5 is an aromatic or heteroaromatic ring, as described herein; and

(b) R_2 is selected from the group consisting of (i) alkyl; (ii) an aromatic or heteroaromatic ring; (iii) an aliphatic or heteroaliphatic ring; (iv) an amine; (v) a ketone; (vi) a carboxylic acid or ester; (vii) an alcohol or an alkoxyalkyl moiety; (viii) an amide; (ix) a sulfonamide; and (x) a sulfone; and

(c) R_3 is selected from the group consisting of (i) hydrogen; (ii) alkyl; (iii) an aromatic or heteroaromatic ring; (iv) an aliphatic or heteroaliphatic ring; (v) a halogen or trihalomethyl; (vi) an amine; (vii) an amide; (viii) an alcohol or an alkoxyalkyl moiety; (ix) a carboxylic acid or ester; (x) a cyano of formula -CN; and (xi) a sulfonamide;

(d) p and q are each independently 0, 1, 2, or 3; and

(e) K and L are each independently selected from the gruop consisting of hydrogen, alkyl, and K and L taken together form a three-membered, four-membered, five-membered, or six-membered aliphatic ring.

10. The compound of claim 8 or claim 9, wherein R_1 is selected from the group consisting of hydrogen, alkyl, aromatic or heteroaromatic ring, and aliphatic or heteroaliphatic ring.

11. The compound of claim 10, wherein R_1 is selected from the group consisting of hydrogen, methyl, *n*-propyl, *i*-propyl, *n*-butyl, isobutyl, *sec*-butyl, and *t*-butyl.

12. The compound of claim 8 or claim 9, wherein R_2 is selected from the group consisting of hydrogen, alkyl, and halogen.

13. The compound of claim 8 or claim 9, wherein R_4 and R_5 are each independently selected from the group consisting of hydrogen, alkyl, aromatic or heteroaromatic ring, and aliphatic or heteroaliphatic ring.

14. The compound of claim 13 wherein R_4 and R_5 are each independently selected from the group consisting of hydrogen, methyl, phenyl, pyridin-2-yl, pyridin-3-yl, pyridin-4-yl, 3-methyl-pyridin-2-yl, 4-methyl-pyridin-2-yl, 5-trifluoromethyl-pyridin-2-yl, 5-trifluoromethyl-pyridin-3-yl, 4,6-dimethyl-pyridin-3-yl, 2,6-dimethoxy-pyridin-3-yl,

2,3,5,6-tetrafluoro-pyridin-4-yl, quinolin-3-yl, 3-methyl-quinolin-4-yl, isoquinolin-1-yl, isoquinolin-3-yl, benzo[1,3]dioxol-5-yl, 4-methoxy-biphenyl-3-yl, 6-methoxy-biphenyl-3-yl, 6,3'-dimethoxy-biphenyl-3-yl, 9-oxo-9H-fluoren-1-yl, 9-oxo-9H-fluoren-3-yl, 7-acetylamino-9-oxo-9H-fluoren-2-yl, 2'-hydroxy-[1,1';3',1"]terphenyl-5'-yl, 9-ethyl-9H-carbazol-3-yl, and 6-oxo-6H-benzo[c]chromen-2-yl.

15. The compound of claim 14, wherein R_4 and R_5 are each independently selected from the group consisting of 2-trifluoromethyl-phenyl, 3-trifluoromethyl-phenyl, and 4-trifluoromethyl-phenyl.

16. The compound of claim 14 or claim 15, wherein R_3 is selected from the group consisting of hydrogen, alkyl, halogen, and trihalomethyl.

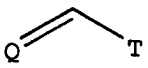
17. The compound of claim 16, wherein R_3 is selected from the group consisting of hydrogen, methyl, and halogen.

18. The compound of claim 8 or claim 9, wherein p and q are each independently 0, 1, 2, or 3.

19. The compound of claim 18, wherein said compound is selected from the group consisting of 2-benzyl-5-tert-butyl-2H-pyrazole-3-carboxylic acid (4-trifluoromethyl-phenyl)-amide, 2-benzyl-5-tert-butyl-2H-pyrazole-3-carboxylic acid quinolin-3-ylamide, 2-benzyl-5-tert-butyl-2H-pyrazole-3-carboxylic acid (2,6-dimethoxy-pyridin-3-yl)-amide, 2-benzyl-5-tert-butyl-2H-pyrazole-3-carboxylic acid (2,3,5,6-tetrafluoro-pyridin-4-yl)-amide, 2-benzyl-5-tert-butyl-2H-pyrazole-3-carboxylic acid (3-methyl-quinolin-4-yl)-amide, 2-benzyl-5-tert-butyl-2H-pyrazole-3-carboxylic acid (4,6-dimethyl-pyridin-3-yl)-amide, 2-benzyl-5-tert-butyl-2H-pyrazole-3-carboxylic acid benzo[1,3]dioxol-5-ylamide, 2-benzyl-5-tert-butyl-2H-pyrazole-3-carboxylic acid (3-trifluoromethyl-phenyl)-amide, 2-benzyl-5-tert-butyl-2H-pyrazole-3-carboxylic acid (2-trifluoromethyl-phenyl)-amide, 2-benzyl-5-tert-butyl-2H-pyrazole-3-carboxylic acid pyridin-2-ylamide, 2-benzyl-5-tert-butyl-2H-pyrazole-3-carboxylic acid isoquinolin-1-ylamide, 2-benzyl-5-tert-butyl-2H-pyrazole-3-carboxylic acid pyridin-4-ylamide, 2-

benzyl-5-tert-butyl-2H-pyrazole-3-carboxylic acid pyridin-3-ylamide, 2-benzyl-5-tert-butyl-2H-pyrazole-3-carboxylic acid (4-methyl-pyridin-2-yl)-amide, 2-benzyl-5-tert-butyl-2H-pyrazole-3-carboxylic acid (3-methyl-pyridin-2-yl)-amide, 2-benzyl-5-tert-butyl-2H-pyrazole-3-carboxylic acid (5-trifluoromethyl-pyridin-2-yl)-amide, 2-benzyl-5-tert-butyl-2H-pyrazole-3-carboxylic acid isoquinolin-3-ylamide, 2-benzyl-5-tert-butyl-2H-pyrazole-3-carboxylic acid (5-trifluoromethyl-pyridin-3-yl)-amide, 2-benzyl-5-tert-butyl-2H-pyrazole-3-carboxylic acid (4-methoxy-biphenyl-3-yl)-amide, 2-benzyl-5-tert-butyl-2H-pyrazole-3-carboxylic acid (9-oxo-9H-fluoren-3-yl)-amide, 2-benzyl-5-tert-butyl-2H-pyrazole-3-carboxylic acid (7-acetylamino-9-oxo-9H-fluoren-2-yl)-amide, 2-benzyl-5-tert-butyl-2H-pyrazole-3-carboxylic acid (6-methoxy-biphenyl-3-yl)-amide, 2-benzyl-5-tert-butyl-2H-pyrazole-3-carboxylic acid (2'-hydroxy-[1,1';3',1"]terphenyl-5'-yl)-amide, 2-benzyl-5-tert-butyl-2H-pyrazole-3-carboxylic acid (9-ethyl-9H-carbazol-3-yl)-amide, 2-benzyl-5-tert-butyl-2H-pyrazole-3-carboxylic acid (9-oxo-9H-fluoren-1-yl)-amide, 2-benzyl-5-tert-butyl-2H-pyrazole-3-carboxylic acid (6-oxo-6H-benzo[c]chromen-2-yl)-amide, 2-benzyl-5-tert-butyl-2H-pyrazole-3-carboxylic acid biphenyl-3-ylamide, 2-benzyl-5-tert-butyl-2H-pyrazole-3-carboxylic acid (6-methoxy-biphenyl-3-yl)-amide, 2-benzyl-5-tert-butyl-2H-pyrazole-3-carboxylic acid (6,3'-dimethoxy-biphenyl-3-yl)-amide, 5-methyl-2-(4-methyl-benzyl)-2H-pyrazole-3-carboxylic acid (4-trifluoromethyl-phenyl)-amide, 5-methyl-2-(4-methyl-benzyl)-2H-pyrazole-3-carboxylic acid (3-trifluoromethyl-phenyl)-amide, 5-methyl-2-(4-chloro-benzyl)-2H-pyrazole-3-carboxylic acid (4-trifluoromethyl-phenyl)-amide, 5-methyl-2-(4-chloro-benzyl)-2H-pyrazole-3-carboxylic acid (3-trifluoromethyl-phenyl)-amide.

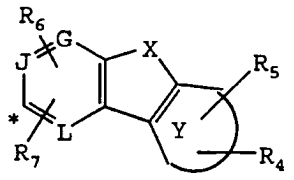
20. An indolinone compound having a structure set forth in formula XI:

(XI) 

wherein

(A) Q is an optionally substituted oxindole moiety bonded with the rest of the molecule through position 3 of the oxindole ring; and

(B) T is a ring moiety having the structure set forth in formula XII:



(XII)

wherein

(a) R_4, R_5, R_6, and R_7 are independently selected from the group consisting of (i) hydrogen; (ii) alkyl; (iii) an aromatic or heteroaromatic ring; (iv) an aliphatic or heteroaliphatic ring; (v) an amine; (vi) a nitro of formula $-NO_2$; (vii) a halogen or trihalomethyl; (viii) a ketone; (ix) a carboxylic acid or ester; (x) an alcohol or an alkoxyalkyl moiety; (xi) an amide; (xii) a sulfonamide; (xiii) an aldehyde; (xiv) a sulfone; and (xv) a thiol or a thioether;

(b) X is selected from the group consisting of NX_{26}, sulfur, SO, SO_2, and oxygen, wherein X_{26} is selected from the group consisting of (i) hydrogen; (ii) alkyl; (iii) an aryl optionally substituted with one, two, or three substituents independently selected from the group consisting of alkyl, alkoxy, halogen, trihalomethyl, carboxylate, nitro, and ester moieties; (iv) a sulfone of formula $-SO_2-X_{27}$, wherein X_{27} is selected from the group consisting of saturated or unsaturated alkyl and five-membered or six-membered aryl or heteroaryl moieties; and (v) an acyl of formula $-C(O)X_{28}$, wherein X_{28} is selected from the group consisting of hydrogen, saturated and unsaturated alkyl, aryl, and a five-membered or six-membered ring moiety;

(c) ring Y is selected from the group consisting of five-membered, six-membered, and seven-membered aromatic, heteroaromatic, or non-aromatic rings, wherein said heteroaromatic ring contains a heteroatom selected from the group consisting of nitrogen, oxygen, and sulfur, and wherein said non-aromatic ring in combination with R_4 optionally forms a carbonyl functionality;

(d) G, J, and L are selected from the group consisting of carbon and nitrogen; and

(e) T is bonded to the rest of the molecule at the position of the ring marked with an asterisk (*) in formula XII.

21. The compound of claim 20, wherein Q is selected from the group consisting of type Q oxindoles.

22. The compound of claim 20, wherein R_4 and R_5 are independently selected from the group consisting of

(i) hydrogen;

(ii) methyl, ethyl, propyl, and butyl groups optionally substituted with substituents selected from the group consisting of halogen, trihalomethyl, cyano, and nitro moieties;

(iii) an amine of formula $-(X_1)_{n1}-NX_2X_3$, wherein X_2 and X_3 are independently selected from the group consisting of hydrogen and optionally substituted saturated alkyl, and X_1 is optionally substituted saturated alkyl, and wherein n1 is 0 or 1, or wherein X_2 and X_3 taken together form a five-membered or a six-membered aliphatic or heteroaliphatic ring, optionally substituted at a ring carbon atom or hetero atom with a substituent selected from the group consisting of methyl, ethyl, propyl, phenyl, and alkoxyphenyl;

(iv) a nitro of formula $-NO_2$;

(v) a halogen or trihalomethyl;

(vi) a ketone of formula $-CO-X_4$, wherein X_4 is selected from the group consiting of methyl, ethyl, propyl, *n*-butyl, and *t*-butyl;

(vii) a carboxylic acid of formula $-(X_6)_{n6}-COOH$ or ester of formula $-(X_7)_{n7}-COO-X_8$, wherein X_6 and X_7 are selected from the group conssiting of a bond, methylene, ethylene, and propylene, and wherein X_8 is selected from the group consisting of methyl and ethyl, and wherein n6 and n7 are independently 0 or 1;

(viii) an amide of formula $-NHCOX_{13}$, or of formula $-CONX_{15}X_{16}$, wherein X_{13}, X_{15}, and X_{16} are each independently selected from the group consisting of hydrogen, methyl, ethyl, propyl, and phenyl;

(ix) $-SO_2NX_{18}X_{19}$, wherein X_{18} and X_{19} are independently selected from the group consisting of hydrogen, methyl, and ethyl;

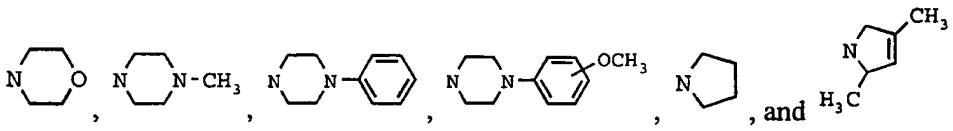
(x) an alcohol of formula $-(X_9)_{n9}-OH$ or an alkoxyalkyl moiety of formula $-(X_{10})_{n10}-O-X_{11}$, wherein X_9, and X_{10} are independently selected form the group

consisting of methylene, ethylene, and propylene, and wherein X_{11} is independently selected from the group consisting of methyl, ethyl, and propyl, and wherein n9 and n10 are independently 0 or 1;

(xi) a sulfone of formula $-(X_{21})_{n21}-SO_2-X_{22}$, wherein X_{22} is selected from the group consisting of hydroxide, saturated or unsaturated alkyl, and five-membered or six-membered aryl or heteroaryl moieties, and wherein X_{21} is saturated alkyl, and wherein n21 is 0 or 1; and

(xii) a thioether of formula $-(X_{24})_{n24}-S-X_{25}$, wherein X_{24} is independently selected from the group consisting of methylene, ethylene, and propylene, and wherein X_{25} is independently selected from the group consisting of methyl, ethyl, propyl, and phenyl, and wherein n24 is 0 or 1.

23. The compound of claim 22, wherein R_4 and R_5 are independently selected from the group consisting of (i) hydrogen; (ii) methyl and ethyl; (iii) an amine of formula $-(X_1)_{n1}-NX_2X_3$, wherein X_2 and X_3 are independently selected from the group consisting of hydrogen methyl and ethyl, and X_1 is methylene or ethylene, and wherein n1 is 0 or 1, or wherein X_2 and X_3 taken together form an optionally substituted ring selected from the group consisting of



; (iv) a nitro of formula $-NO_2$; (v) a halogen; (vi) a ketone of formula $-CO-X_4$, wherein X_4 is selected from the group consiting of methyl and *t*-butyl; (vii) a carboxylic acid of formula $-(X_6)_{n6}-COOH$ or ester of formula $-(X_7)_{n7}-COO-X_8$, wherein X_6 and X_7 are selected from the group conssiting of a bond, methylene, ethylene, and propylene, and wherein X_8 is selected from the group consisting of methyl and ethyl, and wherein n6 and n7 are independently 0 or 1; (viii) an amide of formula $-NHCOX_{13}$, or of formula $-CONX_{15}X_{16}$, wherein X_{13}, X_{15}, and X_{16} are each independently selected from the group consisting of hydrogen, methyl, and phenyl; (ix) a sulfonamide of formula $-SO_2NX_{18}X_{19}$, wherein X_{18} and X_{19} are independently selected from the group consisting of hydrogen, methyl, and ethyl; (x) an alcohol of formula $-(X_9)_{n9}-OH$ or an alkoxyalkyl moiety of formula $-(X_{10})_{n10}-O-X_{11}$, wherein X_9, and X_{10} are independently selected form the group consisting of

methylene, ethylene, and propylene, and wherein X_{11} is independently selected from the group consisting of methyl, ethyl, and propyl, and wherein n9 and n10 are independently 0 or 1; (xi) a sulfone of formula $-SO_2OH$; and (xii) a thioether of formula -S-phenyl.

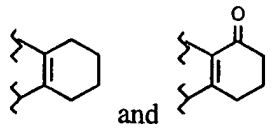
24. The compound of claim 22, wherein R_6 and R_7 are independently selected from the group consisting of (i) hydrogen; (ii) methyl, ethyl, propyl, and butyl groups optionally substituted with halogen, trihalomethyl, cyano, and nitro moieties; (iii) an amine of formula $-(X_1)_{n1}-NX_2X_3$, wherein X_2 and X_3 are independently selected from the group consisting of hydrogen and optionally substituted saturated alkyl, and X_1 is an optionally substituted saturated alkylene, and wherein n1 is 0 or 1; (iv) a halogen or trihalomethyl; and (v) an alcohol of formula $-(X_9)_{n9}-OH$ or an alkoxyalkyl moiety of formula $-(X_{10})_{n10}-O-X_{11}$, wherein X_9, and X_{10} are independently selected form the group consisting of methylene, ethylene, and propylene, and wherein X_{11} is selected from the group consisting of methyl, ethyl, and propyl, and wherein n9 and n10 are independently 0 or 1.

25. The compound of claim 24, wherein R_6 and R_7 are independently selected from the group consisting of (i) hydrogen; (ii) methyl and ethyl; (iii) an amine of formula $-(X_1)_{n1}-NX_2X_3$, wherein X_2 and X_3 are independently selected from the group consisting of hydrogen, methyl, and ethyl, and X_1 is selected from the group consisting of methylene and ethylene, and wherein n1 is 0 or 1; (iv) a halogen; and (v) a hydroxy -OH or an alkoxy moiety of formula $-O-X_{11}$, wherein X_{11} is independently selected form the group consisting of methyl, ethyl, and propyl.

26. The compound of claim 25, wherein Y is a six-membered aromatic or heteroaromatic ring.

27. The compound of claim 25, wherein Y is a six-membered aliphatic or heteroaliphatic ring.

28. The compound of claim 27, wherein Y forms a ring selected from the group consisting of optionally substituted

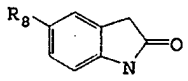
 and .

29. The compound of claim 25, wherein G, J, and L are each independently selected from the group consisting of nitrogen and CH.

30. The compound of claim 25, wherein X is selected from the group consisting of nitrogen, optionally substituted with alkyl sulfur, SO, SO_2, and oxygen.

31. The compound of claim 25, wherein T is selected from the group consisting of type T aldehydes.

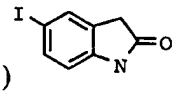
32. An oxindole compound of formula XV

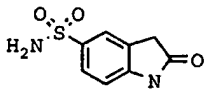


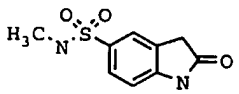
(XV)

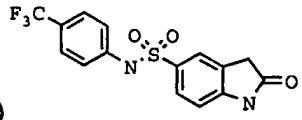
wherein R_8 is selected from the group consisting of (i) saturated alkyl; (ii) an amine; (iii) an iodine; (iv) a ketone of formula $-(X_4)_{n4}-CO-X_5$, wherein X_4 and X_5 are independently alkyl and wherein n4 is 0 or 1; (v) a carboxylic acid or ester; (vi) an amide; and (vii) a sulfonamide.

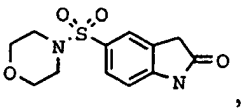
33. The oxindole compound of claim 32, wherein said oxindole compound is selected from the group consisting of

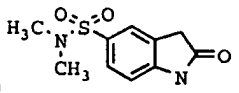
(O-11)  ,

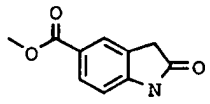
(O-17)  ,

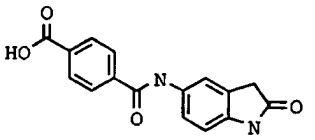
(O-18)  ,

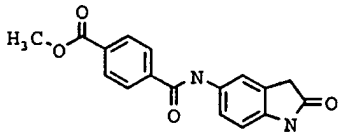
(O-19)  ,

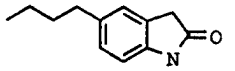
(O-20)  ,

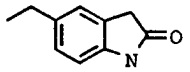
(O-45)  ,

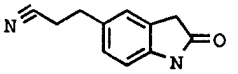
(O-24)  ,

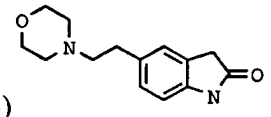
(O-36)  ,

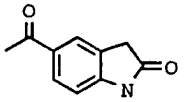
(O-37)  ,

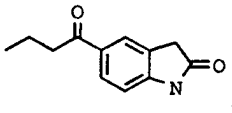
(O-33)  ,

(O-34)  ,

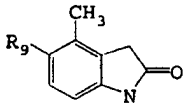
(O-55)  ,

(O-35)  ,

(O-53)  ,

and (O-54)  .

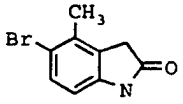
34. An oxindole compound of formula XVI

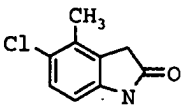


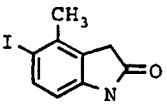
(XVI)

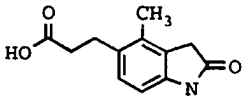
wherein R_9 is selected from the group consisting of (i) an amine; (ii) a nitro of formula - NO_2; (iii) a chlorine, bromine, or iodine; (iv) a ketone; (v) a carboxylic acid or ester; (vi) an amide; and (vii) a sulfonamide.

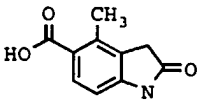
35. The oxindole compound of claim 34, wherein said oxindole compound is selected from the group consisting of

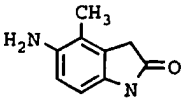
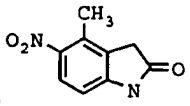
(O-13)  ,

(O-30) 

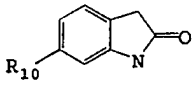
(O-31)  ,

(O-16)  ,

(O-52)  ,

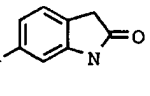
(O-28)  , and (O-29)  .

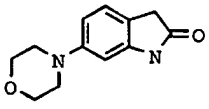
36. An oxindole compound of formula XVII

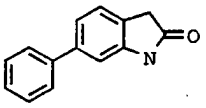
(XVII) 

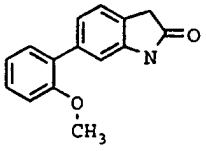
wherein R_{10} is selected from the group consisting of (i) an aromatic or heteroaromatic ring; (ii) an aliphatic or heteroaliphatic ring; (iii) an amine; (iv) a nitro of formula $-NO_2$; (v) a bromine; (vi) a ketone; (vii) a carboxylic acid or an ester; and (viii) a sulfonamide.

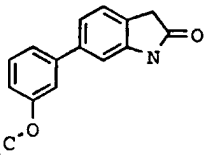
37. The oxindole compound of claim 36, wherein said oxindole compound is selected from the group consisting of

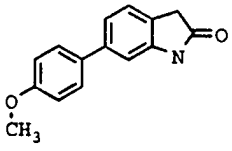
(O-51)  ,

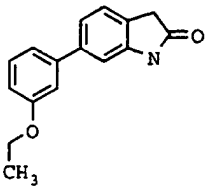
(O-58)  ,

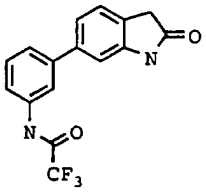
(O-46)  ,

(O-47)  ,

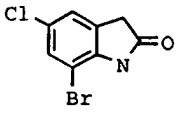
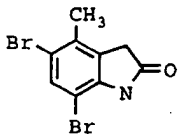
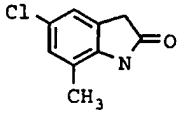
(O-48)  ,

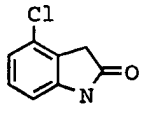
(O-49)  ,

(O-50)  ,

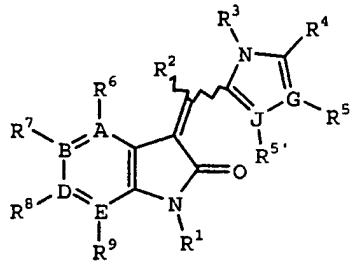
and (O-60)  .

38. An oxindole compound, wherein said oxindole compound is selected from the group consisting of

(O-8)  , (O-32)  , (O-40)  ,

and (O-56)  .

39. An 2-indolinone having the chemical structure:



wherein

(a) A, B, D and E are independently selected from the group consisting of carbon and nitrogen wherein it is understood that, when A, B, D or E is nitrogen, R_6, R_7, R_8 or R_9, respectively, does not exist;

(b) G and J are selected from the group consisting of nitrogen and carbon such that, when G is nitrogen, J is carbon and when J is nitrogen, G is carbon, it being recognized that, when G or J is nitrogen, R_5 or R_5', respectively, does not exist;

(c) R_1 and R_3 are independently selected from the group consisting of hydrogen, alkyl, cycloalkyl, aryl, hydroxy, alkoxy, C-carboxy, O-carboxy, C-amido, C-thioamido, sulfonyl and trihalomethylsulfonyl;

(d) R_2 is selected from the group consisting of hydrogen, alkyl, cycloalkyl, aryl, heteroaryl and halo;

(e) R_4, R_5 and R_5' are independently selected from the group consisting of hydrogen, alkyl, alkenyl, alkynyl, cycloalkyl, aryl, heteroaryl, heteroalicylic, halo, hydroxy, nitro, cyano, alkoxy, aryloxy, C-carboxy, O-carboxy, carbonyl, S-sulfonamido, amino and $NR_{10}R_{11}$ wherein

R_{10} and R_{11} are independently selected from the group consisting of alkyl, cycloalkyl, aryl, carbonyl, sulfonyl, tri-halomethanesulfonyl and, combined, a five-member or a six-member heteroalicyclic ring;

(f) R_6, R_7, R_8 and R_9 are independently selected from the group consisting of hydrogen, alkyl, trihaloalkyl, cycloalkyl, alkenyl, alkynyl, aryl, heteroaryl, heteroalicyclic, hydroxy, alkoxy, aryloxy, thiohydroxy, thioalkoxy, thioaryloxy, sulfinyl, sulfonyl, S-sulfonamido, N-sulfonamido, N-trihalomethanesulfonamido, carbonyl, C-carboxy, O-carboxy, cyano, nitro, halo, cyanato, isocyanato, thiocyanato, isothiocyanato, O-carbamyl, N-carbamyl, O-thiocarbamyl, N-thiocarbamyl, C-amido, N-amido, amino and $-NR_10R_11$ with R_{10} and R_{11} as defined above; and,

(g) R_6 and R_7 or R_7 and R_8 or R_8 and R_9, combined, may form a five- or six-member aromatic, heteroaromatic, alicyclic or heteroalicyclic ring group.

40. The compound of claim 39 wherein A, B, D and E are carbon.

41. The compound of claim 39 wherein at least one of R_1, R_2, and R_3 is hydrogen.

42. The compound of claim 39 wherein R_6, R_7, R_8 and R_9 are independently selected from the group consisting of (i) hydrogen; (ii) unsubstituted lower alkyl; (iii) lower alkyl substituted with a group selected from the group consisting of halo, C-carboxy and $-NR_{10}R_{11}$; (iv) lower alkoxy optionally substituted with a substituent selected from the group consisting of halo, C-carboxy and $NR_{10}R_{11}$; (v) trihalomethyl; (vi) unsubstituted alkenyl; (vii) unsubstituted alkynyl; (viii) aryl optionally substituted with one or more substituents independently selected from the group consisting of unsubstituted lower alkyl, lower alkyl substituted with one or more halo groups, halo, unsubstituted lower alkoxy, C-carboxy, amino, S-sulfonamido or $-NR_{10}R_{11}$; (ix) heteroalicyclic optionally substituted with one or more substituents independently selected from the group consisting of unsubstituted lower alkyl, lower alkyl substituted with one or more halo groups, aldehyde, unsubstituted lower alkyl carbonyl, hydroxy, unsubstituted alkoxy, alkoxy substituted with one or more halo groups, C-carboxy, amino, S-sulfonamido or $-NR_{10}R_{11}$; (x) aryloxy

optionally substituted with one or more substituents independently selected from the group consisting of unsubstituted lower alkyl, trihalomethyl, halo, hydroxy, amino, S-sulfonamido or (xi) $-NR_{10}R_{11}$;

(xii) thiohydroxy;

(xiii) unsubstituted thioalkoxy;

(xiv) thioaryloxy optionally substituted with one or more substituents independently selected from the group consisting of halo, hydroxy, amino, S-sulfonamido or $-NR_{10}R_{11}$; (xv) S-sulfonamido; (xvi) C-carboxy; (xvii) O-carboxy; (xviii) hydroxy; (xix) cyano; (xx) nitro; (xxi) halo; (xxii) C-amido; (xxiii) N-amido; (xxiv) amino; and (xxv) $-NR_{10}R_{11}$.

43. The compound of claim 39 wherein R_{10} is hydrogen and R_{11} is unsubstituted lower alkyl.

44. The compound of claim 39 wherein R_4, R_5 and R_5' are independently selected from the group consisting of hydrogen, unsubstituted lower alkyl, trihalomethyl, lower alkyl susbstituted on the carbon furthest from the point of attachment to the ring with a C-carboxy group, halo, hydroxy, unsubstituted lower alkoxy, O-carboxy, C-carboxy, amino, C-amido, N-amido, nitro, amino, S-sulfonamido and $-NR_{10}R_{11}$.

45. The compound of claim 40 wherein R_1, R_2 and R_3 are hydrogen.

46. The compound of claim claim 42 wherein A, B, D and E are carbon and R_1, R_2 and R_3 are hydrogen.

47. The compound of claim 46 wherein R_{10} is hydrogen and R_{11} is unsubstitued lower alkyl.

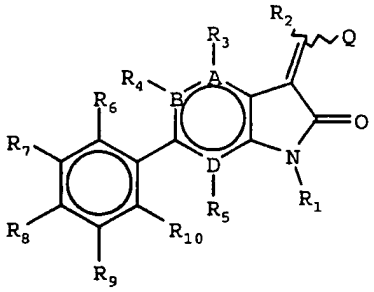
48. The compound of claim 47 wherein R_4 and R_5 and R_5' are independently selected from the group consisting of hydrogen, unsubstituted lower alkyl, lower alkyl substituted at the carbon furthest from the point of attachment to the ring with a C-carboxy

group, trihalomethyl, halo, hydroxy, unsubstituted lower alkoxy, O-carboxy, C-carboxy, amino, C-amido, N-amido, nitro, S-sulfonamido and $-NR_{10}R_{11}$.

49. The compound of claim 47 wherein (a) G is nitrogen; (b) J is carbon; and (c) R_4 and R_5 are hydrogen.

50. The compound of claim 47 wherein (a) G is carbon; (b) J is nitrogen; and (c) R_4 and R_5 are hydrogen.

51. A compound having the chemical structure:



wherein,

(a) A, B and D are independently selected from the group consisting of carbon and nitrogen wherein, when A, B or D is nitrogen, R_3, R_4 or R_5, respectively, does not exist;

(b) R_1 is selected from the group consisting of hydrogen, alkyl, cycloalkyl, aryl, heteroaryl, hydroxy, alkoxy, C-carboxy, O-carboxy, C-amido, C-thioamido, sulfonyl and trihalomethyl- sulfonyl;

(c) R_2 is selected from the group consisting of hydrogen, alkyl, cycloalkyl, aryl, and heteroaryl;

(d) R_3, R_4, R_5, R_6, R_7, R_8, R_9 and R_{10} are independently selected from the group consisting of hydrogen, alkyl, trihaloalkyl, cycloalkyl, alkenyl, alkynyl, aryl, heteroaryl, heteroalicyclic, hydroxy, alkoxy, aryloxy, thiohydroxy, thioalkoxy, thioaryloxy, sulfinyl, sulfonyl, S-sulfonamido, N-sulfonamido, N-trihalomethanesulfonamido, carbonyl, C-carboxy, C-carboxy salt, O-carboxy, carboxyalkyl, carboxyalkyl salt, cyano, azido, nitro,

halo, cyanato, isocyanato, thiocyanato, isothiocyanato, O-carbamyl, N-carbamyl, O-thiocarbamyl, N-thiocarbamyl, C-amido, N-amido, amino and -$NR_{11}R_{12}$;

(e) R_3 and R_4 or R_6 and R_7 or R_7 and R_8 or R_8 and R_9 or R_9 and R_{10} may combine to form a methylenedioxy or an ethylenedioxy group;

(f) Q is selected from the group consisisting of aryl, heteroaryl and fused heteroaryl:cycloalkyl/heteroalicyclic; and,

(g) R_{11} and R_{12} are independently selected from the group consisting of hydrogen, alkyl, cycloalkyl, aryl, carbonyl, acetyl sulfonyl, trihalomethanesulfonyl and, combined, a five-member or a six-member heteroalicyclic ring.

52. The compound, salt or prodrug of claim 51 wherein, R_1 and R_2 are hydrogen.

53. The compound, salt or prodrug of claim 52 wherein, A, B and D are carbon.

54. The compound, salt or prodrug of claim 53 wherein, R_3, R_4, and R_5 are hydrogen.

55. The compound, salt or prodrug of claim 54 wherein R_6, R_7, R_8, R_9, and R_{10} are independently selected from the group consisting of hydrogen and unsubstituted lower alkoxy.

56. The compound salt or prodrug of claim 55 wherein at least one of R_6, R_7, R_8, R_9, or R_{10} is unsubstituted lower alkoxy.

57. The compound, salt or prodrug of claim 56 wherein, when Q is aryl, said aryl group is substituted with one or more groups independently selected from the group consisting of hydrogen, unsubstituted lower alkyl, unsubstituted lower alkoxy and heteroalicyclic.

58. The compound, salt or prodrug of claim 57 wherein said heteroalicyclic group is 4-formylpiperazin-1-yl.

59. The compound, salt or prodrug of claim 56 wherein, when Q is heteroaryl, said heteroaryl group is selected from the group consisting of pyrrol-2-yl, imidazo-4-yl and thiophen-2-yl.

60. The compound, salt or prodrug of claim 56 wherein, when Q is a fused heteroaryl:cycloalkyl/heteroalicyclic group, said fused heteroaryl:cycloalkyl/heteroalicyclic group is 4,5,6,7-tetrahydroindol-2-yl.

61. The compound, salt or prodrug of claim 56 wherein, when Q is heteroaryl or heteroaryl:cycloalkyl/heteroalicyclic, Q is substituted with one or more groups independently selected from the group consisting of hydrogen, unsubstituted lower alkyl, unsubstituted lower alkoxy, carboxy, carboxy salt, carboxyalkyl and carboxyalkyl salt wherein, r in said carboxyalkyl or said carboxyalkyl salt is 1 or 2.

62. A pharmaceutical composition comprising

(i) a physiologically acceptable carrier, diluent, or excipient; and

(ii) a compound according to any one of claims 1, 8, 9, 20, 39, and 51, or a salt thereof.

63. A method of modulating the function of a protein kinase with a indolinone compound according to any one of claims 1, 8, 9, 20, 39, and 51, or a salt thereof, comprising the step of contacting cells expressing said protein kinase with said compound.

64. A method of identifying indolinone compounds that modulate the function of protein kinase, comprising the following steps:

(a) contacting cells expressing said protein kinase with said compound; and

(b) monitoring an effect upon said cells.

65. The method of claim 64, wherein said effect is selected from the group consisting of a change or an absence of a change in cell phenotype, a change or an absence of a change in cell proliferation, a change or absence of a change in the catalytic activity of the said protein kinase, and a change or absence of a change in the interaction between said protein kinase with a natural binding partner, as described herein.

66. The method of claim 65, comprising the following steps:

(a) lysing said cells to render a lysate comprising protein kinase;

(b) adsorbing said protein kinase to an antibody;

(c) incubating said adsorbed protein kinase with a substrate or substrates; and

(d) adsorbing said substrate or substrates to a solid support or antibody;

wherein said step of monitoring said effect on said cells comprises measuring the phosphate concentration of said substrate or substrates.

67. A method for treating a disease related to unregulated tyrosine kinase signal transduction, the method comprising the step of administering to a subject in need thereof a therapeutically effective amount of a compound according to any one of claims 1, 8, 9, 20, 39, and 51.

68. A method of regulating tyrosine kinase signal transduction comprising administering to a subject a therapeutically effective amount of a compound according to any one of claims 1, 8, 9, 20, 39, and 51.

69. A method of preventing or treating an abnormal condition in an organism, wherein said abnormal condition is associated with an aberration in a signal transduction pathway characterized by an interaction between a protein kinase and a natural binding partner, wherein said method comprises the following steps:

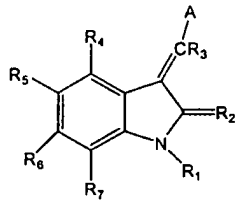
(a) administering a compound of any one of claims of 1, 8, 9, 20, 39, and 51; and

(b) promoting or disrupting the abnormal interaction.

70. The method of claim 69, wherein said organism is a mammal.

71. The method of claim 69, wherein said abnormal condition is selected from the group consisting of cancer, endometriosis, arthritis, ocular neovascularization, solid tumor growth and metastases, excessive scarring during wound healing, rheumatoid arthritis, autoimmune disorders, and transplant rejection.

72. A method of modulating abnormal cell proliferation, comprising administering to a patient in need of such treatment a pharmaceutically acceptable composition comprising a therapeutically effective amount of one or more indolinone compounds of Formula XVIII:



(XVIII)

wherein,

R_1 is H or alkyl;

R_2 is O or S;

R_3 is H;

R_4, R_5, R_6, and R_7 are each independently selected from the group consisting of hydrogen alkyl, alkoxy, aryl, aryloxy, alkaryl, alkaryloxy, halogen, trihalomethyl, S(O)R, SO_2NRR', SO_3R, SR, NO_2, NRR', OH, CN, C(O)R, OC(O)R, NHC(O)R, $(CH_2)_nCO_2R$, CONRR', and $(CH_2)_nONRR'$;

A is selected from the group consisting of a 4,5,6,7-tetrahydroindole and a five-membered heteroaryl ring, wherein said five-membered ring is selected from the group consisting of thiophene, pyrrole, pyrazole, imidazole, 1,2,3-triazole, 1,2,4-triazole, oxazole, isoxazole, thiazole, isothiazole, 2-sulfonylfuran, 4-alkylfuran, 1,2,3-oxadiazole, 1,2,4-oxadiazole, 1,2,5-oxadiazole, 1,3,4-oxadiazole, 1,2,3,4,-oxatriazole, 1,2,3,5-oxatriazole, 1,2,3-thiadiazole, 1,2,4-thiadiazole, 1,2,5-thiadiazole, 1,3,4-thiadaizole, 1,2,3,4-thiatriazole, 1,2,3,5-thiatriazole, and tetrazole, wherein said five-membered ring and said tetrahydroindole are optionally substituted with one or more substituents selected

from the group consisting of alkyl, alkoxy, aryl, aryloxy, alkaryl, alkaryloxy, halogen, trihalomethyl, S(O)R, SO_2NRR', SO_3R, SR, NO_2, NRR', OH, CN, C(O)R, OC(O)R, NHC(O)R, $(CH_2)_nCO_2R$, CONRR', and $(CH_2)_nONRR'$;

n is 0-3;

R is selected from the group consisting of H, alkyl, and aryl; and

R' is selected from the group consisting of H, alkyl, and aryl, wherein said alkyl is optionally substituted with a six-membered heteroaliphatic ring, and wherein said six-membered ring is optionally substituted at one or more positions with substituents selected from the group consisting of alkyl, alkoxy, halogen, trihalomethyl, NO_2, and $(CH_2)_nCO_2R$.

73. The method of claim 72, wherein said A is selected from the group consisting of thiophene, pyrole, and 4,5,6,7-tetrahydroindole, optionally substituted with one or more substituents selected from the group consisting of alkyl, alkoxy, aryl, aryloxy, alkaryl, alkaryloxy, halogen, trihalomethyl, S(O)R, SO_2NRR', SO_3R, SR, NO_2, NRR', OH CN, C(O)R, OC(O)R, NHC(O)R, $(CH_2)_nCO_2R$, CONRR', and $(CH_2)_nONRR$;

n is 0-3;

R is selected from the group consisting of H, alkyl, and aryl; and

R' is selected from the group consisting of H, alkyl, and aryl, wherein said alkyl is optionally substituted with a six-membered heteroaliphatic ring, and wherein said six-membered ring is optionally substituted at one or more positions with substituents selected from the group consisting of alkyl, alkoxy, halogen, trihalomethyl, NO_2, and $(CH_2)_nCO_2R$.

74. The method of claim 72,

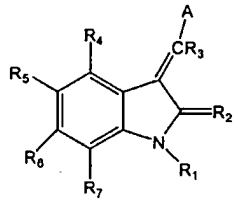
(a) wherein said indolinone compounds of Formula XVIII are selected from the group consisting of the *E* or *Z* isomer of 5-amino-3-(3,5-diethyl-1H-pyrrol-2-ylmethylene)-1,3-dihydro-indol-2-one, the *E* or *Z* isomer of 4-methyl-3-(3-methyl-thiophen-2-ylmethylene)-1,3-dihydro-indol-2-one, the *E* or *Z* isomer of 5-chloro-3-(3,5-dimethyl-1H-pyrrol-2-ylmethylene)-1,3-dihydro-indol-2-one, the *E* or *Z* isomer of 3-[4-methyl-5-(2-oxo-1,2-dihydro-indol-3-ylidenemethyl)-H-pyrrol-3-yl]-propionic acid, 3-[2,4-dimethyl-5-(2-oxo-1,2-dihydro-indol-3-ylidenemethyl)-1H-pyrrol-3-yl]-propionic acid, the *E* or *Z* isomer of N-(2-Morpholin-4-yl-ethyl)-3-[2-(2-oxo-1,2-dihydro-indol-3-ylidenemethyl)-4,5,6,7-tetrahydro-1H-indol-3-yl]-propionamide, and the *E* or *Z* isomer of

3-[2-(2-Oxo-1,2-dihydro-indol-3-ylidenemethyl)-4,5,6,7-tetrahydro-1H-indol-3-yl]-propionic acid;

(b) wherein said composition further comprises one or more pharmaceutically acceptable excipients in a formulation, wherein said formulation is selected from the group consisting of a parenteral, an oral, and a topical formulation; and

(c) wherein said effective amount comprises 1 to 1000 $mg/m^2/day$.

75. A method of modulating the activity of VEGF, FGF, or PDGF on cells *in vivo* or *in vitro*, comprising administering to said cells a pharmaceutically acceptable composition comprising a therapeutically effective amount of one or more indolinone compounds of Formula XVIII:



(XVIII)

R_1 is H or alkyl;

R_2 is O or S;

R_3 is H;

R_4, R_5, R_6, and R_7 are each independently selected from the group consisting of hydrogen alkyl, alkoxy, aryl, aryloxy, alkaryl, alkaryloxy, halogen, trihalomethyl, S(O)R, SO_2NRR', SO_3R, SR, NO_2, NRR', OH, CN, C(O)R, OC(O)R, NHC(O)R, $(CH_2)_nCO_2R$, CONRR', and $(CH_2)_nONRR'$;

A is selected from the group consisting of a 4,5,6,7-tetrahydroindole and a five-membered heteroaryl ring, wherein said five-membered ring is selected from the group consisting of thiophene, pyrrole, pyrazole, imidazole, 1,2,3-triazole, 1,2,4-triazole, oxazole, isoxazole, thiazole, isothiazole, 2-sulfonylfuran, 4-alkylfuran, 1,2,3-oxadiazole, 1,2,4-oxadiazole, 1,2,5-oxadiazole, 1,3,4-oxadiazole, 1,2,3,4,-oxatriazole, 1,2,3,5-oxatriazole, 1,2,3-thiadiazole, 1,2,4-thiadiazole, 1,2,5-thiadiazole, 1,3,4-thiadaizole, 1,2,3,4-thiatriazole, 1,2,3,5-thiatriazole, and tetrazole, wherein said five-membered ring and said tetrahydroindole are optionally substituted with one or more substituents selected from the group consisting of alkyl, alkoxy, aryl, aryloxy, alkaryl, alkaryloxy, halogen,

trihalomethyl, S(O)R, SO_2NRR', SO_3R, SR, NO_2, NRR', OH, CN, C(O)R, OC(O)R, NHC(O)R, $(CH_2)_nCO_2R$, CONRR', and $(CH_2)_nONRR'$;

n is 0-3;

R is selected from the group consisting of H, alkyl, and aryl; and

R' is selected from the group consisting of H, alkyl, and aryl, wherein said alkyl is optionally substituted with a six-membered heteroaliphatic ring, and wherein said six-membered ring is optionally substituted at one or more positions with substituents selected from the group consisting of alkyl, alkoxy, halogen, trihalomethyl, NO_2, and $(CH_2)_nCO_2R$.

76. The method of claim 75, wherein said A is selected from the group consisting of thiophene, pyrrole, and 4,5,6,7-tetrahydroindole, optionally substituted with one or more substituents selected from the group consisting of alkyl, alkoxy, aryl, aryloxy, alkaryl, alkaryloxy, halogen, trihalomethyl, S(O)R, SO_2NRR', SO_3R, SR, NO_2, NRR', OH CN, C(O)R, OC(O)R, NHC(O)R, $(CH_2)_nCO_2R$, CONRR', and $(CH_2)_nONRR$;

n is 0-3;

R is selected from the group consisting of H, alkyl, and aryl; and

R' is selected from the group consisting of H, alkyl, and aryl, wherein said alkyl is optionally substituted with a six-membered heteroaliphatic ring, and wherein said six-membered ring is optionally substituted at one or more positions with substituents selected from the group consisting of alkyl, alkoxy, halogen, trihalomethyl, NO_2, and $(CH_2)_nCO_2R$.

77. The method of claim 75,

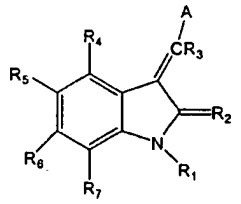
(a) wherein said indolinone compounds of Formula XVIII are independently selected from the group consisting of the *E* or *Z* isomer of 5-amino-3-(3,5-diethyl-1H-pyrrol-2-ylmethylene)-1,3-dihydro-indol-2-one, the *E* or *Z* isomer of 4-methyl-3-(3-methyl-thiophen-2-ylmethylene)-1,3-dihydro-indol-2-one, the *E* or *Z* isomer of 5-chloro-3-(3,5-dimethyl-1H-pyrrol-2-ylmethylene)-1,3-dihydro-indol-2-one, the *E* or *Z* isomer of 3-[4-methyl-5-(2-oxo-1,2-dihydro-indol-3-ylidenemethyl)-H-pyrrol-3-yl]-propionic acid, 3-[2,4-dimethyl-5-(2-oxo-1,2-dihydro-indol-3-ylidenemethyl)-1H-pyrrol-3-yl]-propionic acid, the *E* or *Z* isomer of N-(2-Morpholin-4-yl-ethyl)-3-[2-(2-oxo-1,2-dihydro-indol-3-ylidenemethyl)-4,5,6,7-tetrahydro-1H-indol-3-yl]-propionamide, and the *E* or *Z* isomer of

3-[2-(2-Oxo-1,2-dihydro-indol-3-ylidenemethyl)-4,5,6,7-tetrahydro-1H-indol-3-yl]-propionic acid;

(b) wherein said composition further comprises one or more pharmaceutically acceptable excipients in a formulation, wherein said formulation is selected from the group consisting of a parenteral, an oral, and a topical formulation; and

(c) wherein said effective amount comprises 1 to 1000 $mg/m^2/day$.

78. A method of modulating tyrosine kinase signal transduction, comprising administering to a patient in need of such treatment a pharmaceutically acceptable composition comprising a therapeutically effective amount of one or more indolinone compounds of Formula XVIII:



(XVIII)

R_1 is H or alkyl;

R_2 is O or S;

R_3 is H;

R_4, R_5, R_6, and R_7 are each independently selected from the group consisting of hydrogen alkyl, alkoxy, aryl, aryloxy, alkaryl, alkaryloxy, halogen, trihalomethyl, S(O)R, SO_2NRR', SO_3R, SR, NO_2, NRR', OH, CN, C(O)R, OC(O)R, NHC(O)R, $(CH_2)_nCO_2R$, CONRR', and $(CH_2)_nONRR'$;

A is selected from the group consisting of a 4,5,6,7-tetrahydroindole and a five-membered heteroaryl ring, wherein said five-membered ring is selected from the group consisting of thiophene, pyrrole, pyrazole, imidazole, 1,2,3-triazole, 1,2,4-triazole, oxazole, isoxazole, thiazole, isothiazole, 2-sulfonylfuran, 4-alkylfuran, 1,2,3-oxadiazole, 1,2,4-oxadiazole, 1,2,5-oxadiazole, 1,3,4-oxadiazole, 1,2,3,4,-oxatriazole, 1,2,3,5-oxatriazole, 1,2,3-thiadiazole, 1,2,4-thiadiazole, 1,2,5-thiadiazole, 1,3,4-thiadaizole, 1,2,3,4-thiatriazole, 1,2,3,5-thiatriazole, and tetrazole, wherein said five-membered ring and said tetrahydroindole are optionally substituted with one or more substituents selected from the group consisting of alkyl, alkoxy, aryl, aryloxy, alkaryl, alkaryloxy, halogen,

trihalomethyl, S(O)R, SO_2NRR', SO_3R, SR, NO_2, NRR', OH, CN, C(O)R, OC(O)R, NHC(O)R, $(CH_2)_nCO_2R$, CONRR', and $(CH_2)_nONRR'$;

n is 0-3;

R is selected from the group consisting of H, alkyl, and aryl; and

R' is selected from the group consisting of H, alkyl, and aryl, wherein said alkyl is optionally substituted with a six-membered heteroaliphatic ring, and wherein said six-membered ring is optionally substituted at one or more positions with substituents selected from the group consisting of alkyl, alkoxy, halogen, trihalomethyl, NO_2, and $(CH_2)_nCO_2R$.

79. The method of claim 78, wherein said A is selected from the group consisting of thiophene, pyrrole, and 4,5,6,7-tetrahydroindole, optionally substituted with one or more substituents selected from the group consisting of alkyl, alkoxy, aryl, aryloxy, alkaryl, alkaryloxy, halogen, trihalomethyl, S(O)R, SO_2NRR', SO_3R, SR, NO_2, NRR', OH CN, C(O)R, OC(O)R, NHC(O)R, $(CH_2)_nCO_2R$, CONRR', and $(CH_2)_nONRR$;

n is 0-3;

R is selected from the group consisting of H, alkyl, and aryl; and

R' is selected from the group consisting of H, alkyl, and aryl, wherein said alkyl is optionally substituted with a six-membered heteroaliphatic ring, and wherein said six-membered ring is optionally substituted at one or more positions with substituents selected from the group consisting of alkyl, alkoxy, halogen, trihalomethyl, NO_2, and $(CH_2)_nCO_2R$.

80. The method of claim 78,

(a) wherein said indolinone compounds of Formula XVIII are independently selected from the group consisting of the *E* or *Z* isomer of 5-amino-3-(3,5-diethyl-1H-pyrrol-2-ylmethylene)-1,3-dihydro-indol-2-one, the *E* or *Z* isomer of 4-methyl-3-(3-methyl-thiophen-2-ylmethylene)-1,3-dihydro-indol-2-one, the *E* or *Z* isomer of 5-chloro-3-(3,5-dimethyl-1H-pyrrol-2-ylmethylene)-1,3-dihydro-indol-2-one, the *E* or *Z* isomer of 3-[4-methyl-5-(2-oxo-1,2-dihydro-indol-3-ylidenemethyl)-H-pyrrol-3-yl]-propionic acid, 3-[2,4-dimethyl-5-(2-oxo-1,2-dihydro-indol-3-ylidenemethyl)-1H-pyrrol-3-yl]-propionic acid, the *E* or *Z* isomer of N-(2-Morpholin-4-yl-ethyl)-3-[2-(2-oxo-1,2-dihydro-indol-3-ylidenemethyl)-4,5,6,7-tetrahydro-1H-indol-3-yl]-propionamide, and the *E* or *Z* isomer of

3-[2-(2-Oxo-1,2-dihydro-indol-3-ylidenemethyl)-4,5,6,7-tetrahydro-1H-indol-3-yl]-propionic acid;

(b) wherein said composition further comprises one or more pharmaceutically acceptable excipients in a formulation, wherein said formulation is selected from the group consisting of a parenteral, an oral, and a topical formulation; and

(c) wherein said effective amount comprises 1 to 1000 mg/m^2/day.

81. A method of identifying one or more indolinone compounds of Formula XVIII that inhibit growth factor-stimulated cell proliferation comprising the following steps:

(a) contacting cells with said one or more indolinone compounds;

(b) contacting said cells with one or more growth factors selected from the group consisting of VEGF, PDGF, and FGF; and

(c) monitoring an effect upon said cells.

82. The method of claim 81, wherein said growth factor is VEGF and said cells are endothelial cells.

83. The method of claim 81, wherein said growth factor is PDGF and said cells are smooth muscle cells.

84. The method of claim 81, wherein said growth factor is FGF and said cells are endothelial cells.

85. A method of identifying one or more indolinone compounds of Formula XVIII that are active in an adjuvant arthritis model in rats comprising the following steps:

(a) administering said one or more indolinone compounds to said rats; and

(b) monitoring an effect upon said rats.

86. The method of claim 85, wherein said one or more compounds are administered at a concentration of 1 to 1000 mg/m^2/day.

87. A method of modulating abnormal cell proliferation, comprising administering to a patient in need of such treatment a pharmaceutically acceptable composition comprising a therapeutically effective amount of said one or more compounds identified by the method of either of claims 81 or 85.

88. The method of claim 87, wherein said composition further comprises one or more pharmaceutically acceptable excipients in a formulation, wherein said formulation is selected from the group consisting of a parenteral, an oral, and a topical formulation.

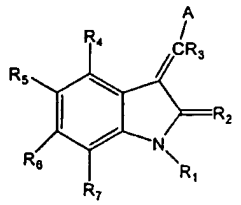
89. A method of modulating the activity of VEGF, FGF, or PDGF on cells *in vivo* or *in vitro*, comprising administering to a patient in need of such treatment a pharmaceutically acceptable composition comprising a therapeutically effective amount of said one or more compounds identified by the method of either of claims 81 or 85.

90. The method of claim 89, wherein said composition further comprises one or more pharmaceutically acceptable excipients in a formulation, wherein said formulation is selected from the group consisting of a parenteral, an oral, and a topical formulation.

91. A method of modulating tyrosine kinase signal transduction, comprising administering to a patient in need of such treatment a pharmaceutically acceptable composition comprising a therapeutically effective amount of said one or more compounds identified by the method of either of claims 81 or 85.

92. The method of claim 91, wherein said composition further comprises one or more pharmaceutically acceptable excipients in a formulation, wherein said formulation is selected from the group consisting of a parenteral, an oral, and a topical formulation.

93. A method of treating or preventing an abnormal condition by administering to a patient in need of such treatment a pharmaceutically acceptable composition comprising a therapeutically effective amount of one or more indolinone compounds of Formula XVIII:



(XVIII)

R_1 is H or alkyl;

R_2 is O or S;

R_3 is H;

R_4, R_5, R_6, and R_7 are each independently selected from the group consisting of hydrogen alkyl, alkoxy, aryl, aryloxy, alkaryl, alkaryloxy, halogen, trihalomethyl, S(O)R, SO_2NRR', SO_3R, SR, NO_2, NRR', OH, CN, C(O)R, OC(O)R, NHC(O)R, $(CH_2)_nCO_2R$, CONRR', and $(CH_2)_nONRR'$;

A is selected from the group consisting of a 4,5,6,7-tetrahydroindole and a five-membered heteroaryl ring, wherein said five-membered ring is selected from the group consisting of thiophene, pyrrole, pyrazole, imidazole, 1,2,3-triazole, 1,2,4-triazole, oxazole, isoxazole, thiazole, isothiazole, 2-sulfonylfuran, 4-alkylfuran, 1,2,3-oxadiazole, 1,2,4-oxadiazole, 1,2,5-oxadiazole, 1,3,4-oxadiazole, 1,2,3,4,-oxatriazole, 1,2,3,5-oxatriazole, 1,2,3-thiadiazole, 1,2,4-thiadiazole, 1,2,5-thiadiazole, 1,3,4-thiadaizole, 1,2,3,4-thiatriazole, 1,2,3,5-thiatriazole, and tetrazole, wherein said five-membered ring and said tetrahydroindole are optionally substituted with one or more substituents selected from the group consisting of alkyl, alkoxy, aryl, aryloxy, alkaryl, alkaryloxy, halogen, trihalomethyl, S(O)R, SO_2NRR', SO_3R, SR, NO_2, NRR', OH, CN, C(O)R, OC(O)R, NHC(O)R, $(CH_2)_nCO_2R$, CONRR', and $(CH_2)_nONRR'$;

n is 0-3;

R is selected from the group consisting of H, alkyl, and aryl; and

R' is selected from the group consisting of H, alkyl, and aryl, wherein said alkyl is optionally substituted with a six-membered heteroaliphatic ring, and wherein said six-membered ring is optionally substituted at one or more positions with substituents selected from the group consisting of alkyl, alkoxy, halogen, trihalomethyl, NO_2, and $(CH_2)_nCO_2R$; and

wherein said abnormal condition is selected from the group consisting of arthritis, endometriosis, ocular neovascularization, solid tumor growth and metastases, and excessive scarring during wound healing.

94. The method of claim 93, wherein said A is selected from the group consisting of thiophene, pyrrole, and 4,5,6,7-tetrahydroindole, optionally substituted with one or more substituents selected from the group consisting of alkyl, alkoxy, aryl, aryloxy, alkaryl, alkaryloxy, halogen, trihalomethyl, S(O)R, SO_2NRR', SO_3R, SR, NO_2, NRR', OH CN, C(O)R, OC(O)R, NHC(O)R, $(CH_2)_nCO_2R$, CONRR', and $(CH_2)_nONRR$;

n is 0-3;

R is selected from the group consisting of H, alkyl, and aryl; and

R' is selected from the group consisting of H, alkyl, and aryl, wherein said alkyl is optionally substituted with a six-membered heteroaliphatic ring, and wherein said six-membered ring is optionally substituted at one or more positions with substituents selected from the group consisting of alkyl, alkoxy, halogen, trihalomethyl, NO_2, and $(CH_2)_nCO_2R$.

95. The method of claim 94, wherein said indolinone compounds of Formula XVIII are independently selected from the group consisting of Compound AV-002, Compound AV-003, and Compound AV-004.

96. The method of claim 93, wherein said abnormal condition is endometriosis.

97. The method of claim 93, wherein said disease is arthritis.

98. The method of claim 93, wherein said composition further comprises one or more pharmaceutically acceptable excipients in a formulation.

99. The method of claim 93, wherein said formulation is selected from the group consisting of a parenteral, an oral, and a topical formulation.

100. A method of treating or preventing an abnormal condition by administering to a patient in need of such treatment a pharmaceutically acceptable

composition comprising a therapeutically effective amount of said one or more compounds identified by the method of either of claims 64, 81, or 85, wherein said abnormal condition is selected from the group consisting of arthritis, endometriosis, ocular neovascularization, solid tumor growth and metastases, and excessive scarring during wound healing.

101. The method of claim 100, wherein said abnormal condition is endometriosis.

102. The method of claim 100, wherein said disease is arthritis.

103. The method of claim 100, wherein said composition further comprises one or more pharmaceutically acceptable excipients in a formulation.

104. The method of claim 100, wherein said formulation is selected from the group consisting of a parenteral, an oral, and a topical formulation.

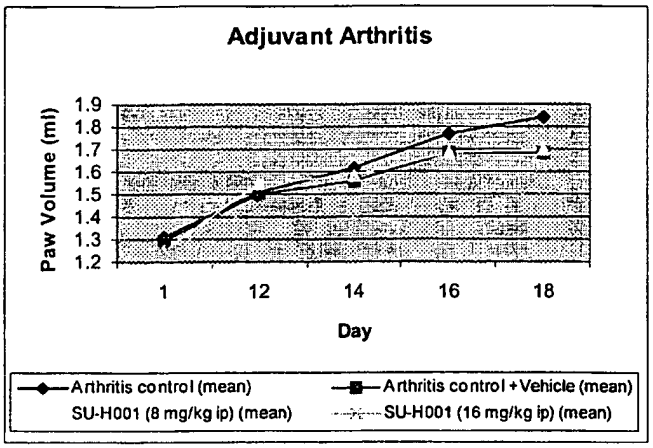


Figure 1

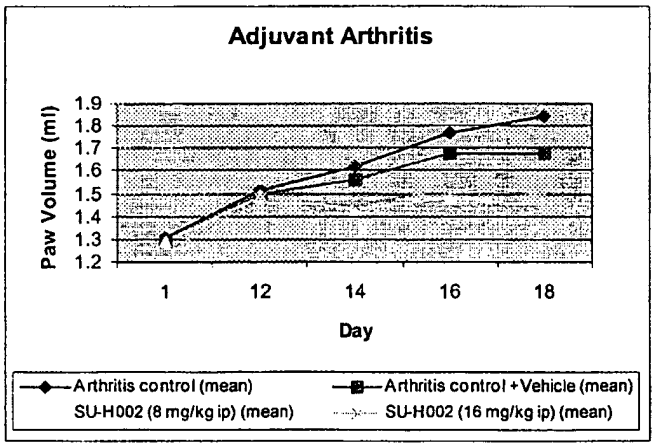


Figure 2

Figure 3

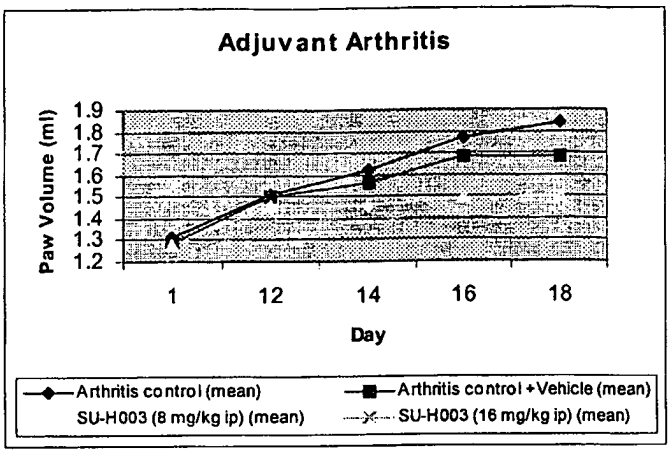
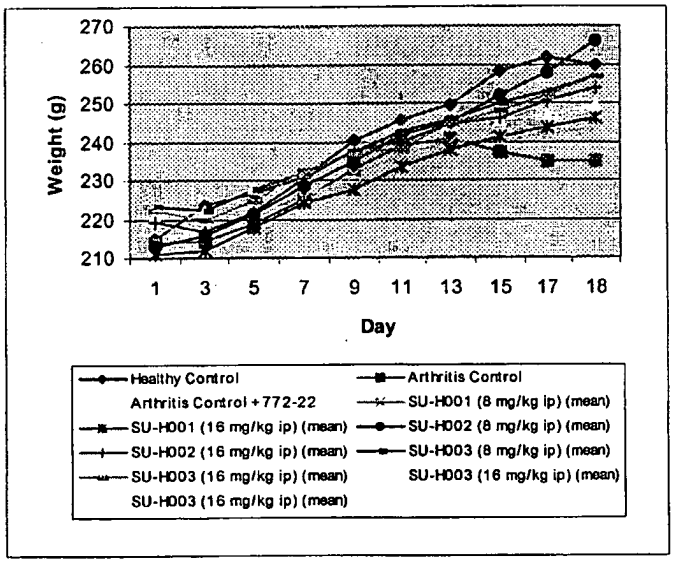


Figure 4



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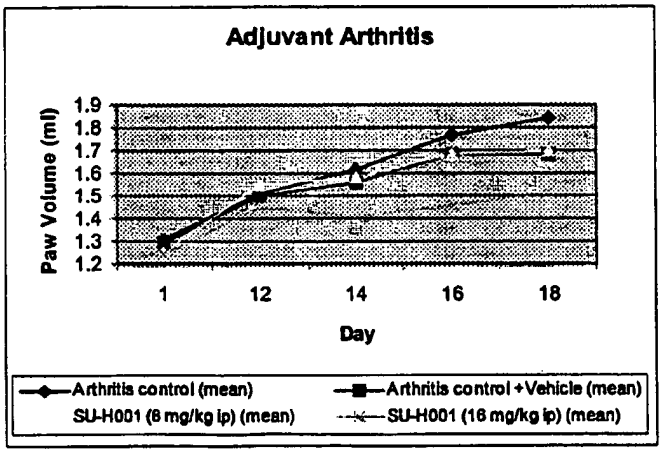
(54) Title: HETEROCYCLIC CLASSES OF COMPOUNDS FOR THE MODULATING TYROSINE PROTEIN KINASE

(57) Abstract

The invention relates to certain indolinone-based and pyrazolylamide-based compounds, their method of synthesis, and combinatorial libraries consisting of the compounds. The invention also relates to methods of modulating the function of protein kinases using these compounds and methods of treating diseases by modulating the function of protein kinases and related signal transduction pathways.



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INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 99/06468

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C07D209/34 C07D403/06 C07D409/06 A61K31/40 A61K31/415

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
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Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	WO 98 50356 A (SUGEN INC) 12 November 1998 (1998-11-12)	1-7,62
X	* overlap of chemical formula, see claim 1, definition of Q = 3,5 * the whole document	39-61

X	WO 98 07695 A (HIRTH KLAUS PETER ;SHAWVER LAURA KAY (US); SUGEN INC (US); TANG PE) 26 February 1998 (1998-02-26)	1-7,62
Y	* overlap of chemical formula, see e.g. claim 6, definition of R8 and R9, claim 10, defintion of R5 and R6 etc. * the whole document	39-61

Y	WO 94 14808 A (ERBA CARLO SPA) 7 July 1994 (1994-07-07) cited in the application the whole document	1-7,62

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[X] Further documents are listed in the continuation of box C.

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Date of the actual completion of the international search	Date of mailing of the international search report
16 November 1999	10. 12. 99

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INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 99/06468

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	SPADA A P ET AL: "SMALL MOLECULE INHIBITORS OF TYROSINE KINASE ACTIVITY" EXPERT OPINION ON THERAPEUTIC PATENTS, vol. 5, no. 8, 1 January 1995 (1995-01-01), pages 805-817, XP000567587 ISSN: 1354-3776	1-7,62
Y	* see page 812, Fig.2, page 813, Tab.1 * the whole document	8-19

Y	WO 96 00226 A (PHARMACIA SPA ;BUZZETTI FRANCO (IT); BRASCA GABRIELLA MARIA (IT);) 4 January 1996 (1996-01-04)	1-7,62
X	the whole document	39-50

Y	WO 96 16964 A (PHARMACIA SPA ;BUZZETTI FRANCO (IT); BRASCA GABRIELLA MARIA (IT);) 6 June 1996 (1996-06-06)	1-7,62
Y	the whole document	39-50

Y	WO 91 13055 A (ERBA CARLO SPA) 5 September 1991 (1991-09-05) the whole document	1-7,62

Y	WO 92 07830 A (PFIZER) 14 May 1992 (1992-05-14)	1-7,62
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5

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 99/06468

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	LEVITZKI A ET AL: "TYROSINE KINASE INHIBITION: AN APPROACH TO DRUG DEVELOPMENT" SCIENCE,US,AMERICAN ASSOCIATION FOR THE ADVANCEMENT OF SCIENCE,, vol. 267, March 1995 (1995-03), page 1782-1788 XP002048355 ISSN: 0036-8075 * see p.1784/1785, ex.AG1112 * the whole document ---	8-19,62
Y	GAZIT,A. ET AL.: "Tyrphostins. 2. Heterocyclic and alpha-Substituted Benzylidenmalonitirle Tyrphostins as Poitent Inhibitors of EGRF Receptor and ErbB2/neu Tyrosine Kinases" J.MED.CHEM., vol. 34, no. 6, June 1991 (1991-06), pages 1896-1907, XP000472938 WASHINGTON * see p.1897, table 1, ex. 11 * the whole document ---	8-19,62
Y,P	SUN,LO. ET AL.: "Synthesis and Biological Evaluation of 3-Substituted Indolin-2-ones : A novel Class of Tyrosine Kinase Inhibitors That Exhibit Selectivity toward Particualr Receptor Tyrosine Kinases" J.MED.CHEM., vol. 41, no. 14, 2 July 1998 (1998-07-02), pages 2588-2603, XP002122185 WASHINGTON	8-19,62
X,P	* see p.2589, chart 1, p.2591, scheme 4,p.2596, table 5 * the whole document ---	39-50
X,P	WO 98 56376 A (SUGEN INC) 17 December 1998 (1998-12-17) the whole document ---	8-19,62
X	WO 96 40116 A (SUGEN INC) 19 December 1996 (1996-12-19)	39-50,62
Y	* overlap of chemical formula, see claims 7,8,14,20, the definition of the substituent A * the whole document ---	51-61

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5

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 99/06468

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	MOHAMMADI M ET AL: "STRUCTURES OF THE TYROSINE KINASE DOMAIN OF FIBROBLAST GROWTH FACTOR RECEPTOR IN COMPLEX WITH INHIBITORS" SCIENCE,US,AMERICAN ASSOCIATION FOR THE ADVANCEMENT OF SCIENCE,, vol. 276, no. 5314, page 955-960 XP002065235 ISSN: 0036-8075 * see p.956, SU5402 * the whole document ---	39-62
P,X	WO 98 45708 A (CLARY DOUGLAS ;SUGEN INC (US)) 15 October 1998 (1998-10-15) * see p.55, indolines 1 - 6 * the whole document -----	39-50

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 99/06468

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Although claims 63-104 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [X] Claims Nos.: 20-38
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

see FURTHER INFORMATION sheet PCT/ISA/210

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [X] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

1-19,39-62

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[X] No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

Claims Nos.: 20-38

Present claims 8 -19 and 62 relate to an extremely large number of possible compounds with only a small part being fixed (see in particular the definition of p together with K and L). Support within the meaning of Article 6 PCT and/or disclosure within the meaning of Article 5 PCT is to be found, however, for only a very small proportion of the compounds claimed. In the present case, the claims so lack support, and the application so lacks disclosure, that a meaningful search over the whole of the claimed scope is impossible. Consequently, the search has been carried out for those parts of the claims which appear to be supported and disclosed, namely those parts relating to the compounds of example 2, pages 92-94, see also examples of tables 10 and 11 (pages 27-29 e.g . those compounds prepared in these examples and closely related analogues/homologues compounds).

Present claims 1-7 and 62 relate to an extremely large number of possible compounds, with partially completely different chemical character, see claim 6). Support within the meaning of Article 6 PCT and/or disclosure within the meaning of Article 5 PCT is to be found, however, for only a very small proportion of the compounds claimed. In the present case, the claims so lack support, and the application so lacks disclosure, that a meaningful search over the whole of the claimed scope is impossible. Consequently, the search has been carried out for those parts of the claims which appear to be supported and disclosed, namely those parts relating to the compounds of example 1 (pages 90-92 e.g those compounds etc. prepared in these examples and closely related homologous compounds etc.).

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: 1-7,62

   Tricyclic 3-methylene-2-oxindole based compounds of formula I and II

2. Claims: 8-19,62

   Pyrazole-amide based compounds

3. Claims: 20-38,62

   3-Tricylic substituted 3-methylene-2-oxindole based compounds and intermediates for their production

4. Claims: 39-50,62

   3-Monocyclic substituted 3-methylene-2-oxindole based compounds

5. Claims: 51-61,62

   6-Phenyl-3-methylene-2-oxindole based compounds

INTERNATIONAL SEARCH REPORT

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